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THE AMERICAN
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STUDIES IN SECONDARY TRAUMATIC SHOCK

III. Circulatory Failure Due to Adrenalin

JOSEPH ERLANGER and HERBERT S. GASSER

From the Physiological Laboratory of Washington University, St. Louis

Received for publication May 22, 1919

Early in 1917 the English Medical Research Committee, reporting for Bainbridge and Trevan (1), announced that shock can be produced in animals by the continuous injection of adrenalin for 20 minutes or more, at such a rate as to keep the arterial pressure up to that attained by moderate stimulation of sensory nerves. They found that during the injection the arterial pressure rose to a high level, that the systemic venous pressure either was not altered or fell, and that the portal pressure rose and remained at a high level, indicating an obstruction to the flow of blood through the liver. In the course of our investigation of shock we have repeated certain phases of the experiments of Bainbridge and Trevan and as a result have gained considerable information bearing upon the so-called shock-producing action of adrenalin, and upon the action of massive doses of adrenalin in general.

In the course of this investigation information was desired with regard to the effect of interposing a high resistance to the passage of the blood through the liver at the point where the normal peripheral resistance of this organ presumably resides. This led to a study of the effect upon the circulation of injecting into the portal vein toward the liver a suspension of lycopodium spores. This series of experiments forms the basis of the second part of this paper.

THE CIRCULATION AFTER THE CONTINUOUS INJECTION OF MASSIVE DOSES OF ADRENALIN

Methods

The adrenalin, usually consisting of from 6 to 11 cc. of the 1:1000 solution freshly obtained from Parke, Davis and Company, was injected, usually into the femoral vein, in the course of 21 to 29 minutes, an aliquot part entering the circulation rapidly at the beginning of each half-minute. After the first two or three injections the arterial pressure no longer falls in the intervals;—the effect becomes continuous. In a few experiments the injection was made into a mesenteric vein. We were unable to distinguish any clear difference in the response of the organism to the administration of the adrenalin by these different paths. A record was made of the pressure in the carotid artery, and the peripheral resistance was measured by the inflow method (2), (3). In some of the experiments readings of the jugular and portal venous pressures were also made from time to time, by the method previously described. It must be borne in mind in connection with these experiments that the inflow method gives but faint clue to the action of the vasomotor center as long as the adrenalin is exerting its effects peripherally. Dogs alone were used; they were anesthetized with ether. The criteria of shock herein employed have been those described in the first paper of this series (3).

Results

The action of these doses of adrenalin has been so variable that a clear conception of it can be conveyed only by describing and discussing a number of the experiments. An attempt at generalization before such a detailed treatment would surely fail to carry conviction.

Experiment 41 (fig. 1). The first injection, lasting 21 minutes, consisted of about 6 cc. of 1:1000 adrenalin. It raised the arterial pressure from 120 mm. Hg. and held it at about 168 mm. Hg. With the termination of the injection the arterial pressure fell abruptly to 74 mm. Hg., and then to 62, before it recovered slowly to 92 mm. Hg.

The inflow rate (femoral area) was not followed during the injection period, but immediately thereafter it was almost nil. It quickly increased, however, to attain more than twice its initial rate within 5 minutes, and then decreased somewhat more deliberately, finally becoming constant at about half the initial rate. It would seem, therefore, that the adrenalin at first practically stops the blood flow in the femoral area, but that, with the termination of the injection, there develops, almost immediately, a tremendous dilatation. When this has passed

off and the peripheral action of the adrenalin presumably has come to an end, the center seems to be normally active; indeed it is more than normally active, presumably under the stimulus of an abnormally low arterial pressure.

A second injection of 6 cc. adrenalin in the course of 20 minutes raised the arterial pressure to 164 mm. Hg. and caused the heart to become very irregular, *c*. With the completion of the injection the arterial pressure declined somewhat more slowly than after the first injection, but it finally got as low as 40 mm. Hg. The heart here, *d*, seemed to be failing; but about 20 minutes later, at *e*, the heart's action improved and the arterial pressure began to rise.

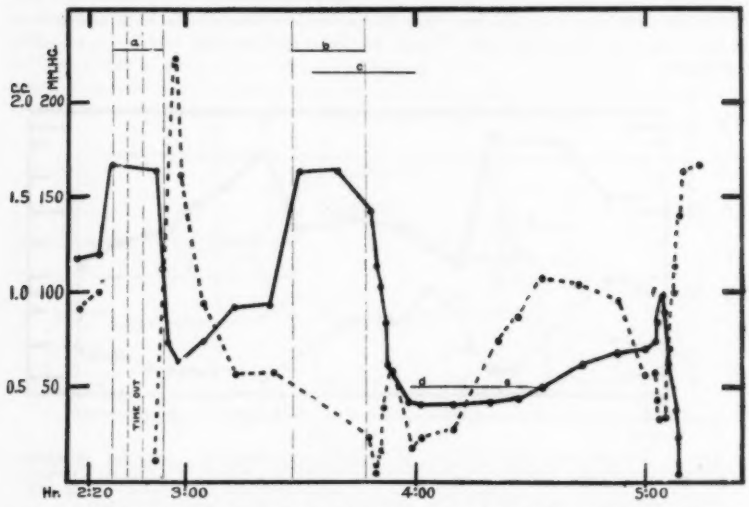

Fig. 1. Experiment 41. Effect of injecting adrenalin. Arterial pressure, —●—●—; femoral inflow, - - ●- - ●- -. *a*, injecting 6.0 cc. adrenalin, 1: 1000; *b*, injecting 6.0 cc. adrenalin, 1: 1000; *c*, heart irregular; *d*, heart seems to be failing; *e*, heart improving; *f*, trachea clamped until animal dies.

This injection, also, practically stopped the femoral inflow. And similarly, the inflow rate, after the termination of the injection, started almost at once to increase; but it soon decreased again to begin a slower ascent. The parts of the inflow curve between the termination of the second injection and about *5:00* may be regarded as picturing *a*, the recovery of the vasoconstrictor mechanism from the peripheral action of the adrenalin (1st ascent); *b*, the stimulation of the center by the low arterial pressure (1st descent); *c*, the giving way of the center under the influence of the low arterial pressure of 40 mm. Hg. (2nd ascent); and finally, *d*, the recovery of the center as the arterial pressure rises (2nd descent), the center here being stimulated by the low arterial pressure to maintain a high grade of constriction. As the animal seemed to be recovering from the second dose the experiment was terminated by clamping the trachea, at *f*. The preliminary as-

phyxial constriction followed by complete failure of the constrictor center is readily discernible in the chart.

In this experiment, therefore, the injection of adrenlin, 1: 1000, at the rate of about 0.04 cc. per second failed to produce shock. The circulation was, however, distinctly damaged by each of the injections, as evidenced by the low arterial pressure and the high tone of the center that finally developed. The experiment gives no clue to the cause of the disturbance, except to indicate that a part of the effect of the second dose probably was exerted through the heart.

Experiment 42 (fig. 2). The first injection (5 cc. of 1: 1000 adrenalin in 22 minutes, dog weighing 6 kilo) raises the arterial pressue from 107 to 140 mm. Hg. After the completion of the injection the arterial pressure falls in 12 minutes to 67 mm. Hg. and then rises more slowly to about 90 mm. Hg. where, practically, it remains.

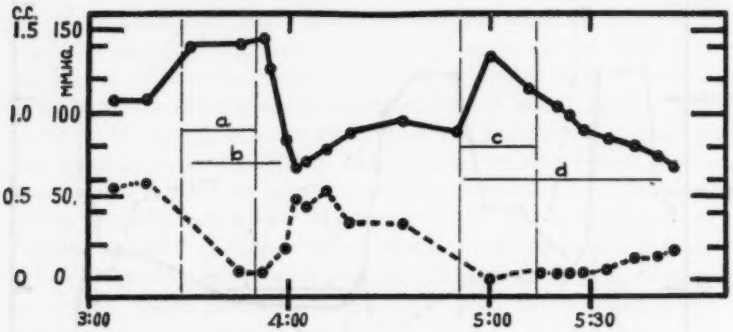

Fig. 2. Experiment 42. Effect of injecting adrenalin. Arterial pressure, —●—●—; femoral inflow, -·-●-·-●-·-. *a*, injecting 5.0 cc. adrenalin, 1: 1000; *b*, heart irregular; *c*, injecting 7.5 cc. adrenalin, 1: 1000; *d*, heart irregular.

During and immediately after the injection, the inflow (femoral) is practically nil, but in 12 minutes it has returned almost to the initial level. Subsequently, however, the inflow rate falls somewhat. As in the preceding experiment, therefore, when the conditions become constant after the injection, the vasomotor tone is high, yet the arterial pressure is somewhat depressed. Evidently the circulation has been hurt; even under the influence of a vasomotor constriction the arterial pressure is subnormal.

The second dose consisted of 7.5 cc. of 1: 1000 adrenalin; it was given in 23 minutes. Although it constricts the arteries even more markedly than did the first dose, yet the arterial pressure does not rise so high; and despite continuation of the constriction, the arterial pressure falls during the terminal part of the injection as well as subsequently. At the time the experiment was terminated the inflow rate was increasing, but still was well below normal, and the arterial pressure was falling. The heart became irregular during the injection and remained so almost to the end. It is doubtful if the fall in pressure can be attributed to this effect of the adrenalin upon the heart, for when the heart eventually became regu-

lar the blood pressure did not improve. Presumably the process would have gone on to death if the experiment could have been continued longer.

In this experiment the cause of the low arterial pressure is not definitely determinable; it probably is not due to the effect upon the heart, and certainly not to peripheral dilatation. By exclusion, therefore, a diminution in blood volume seems to be the disturbing factor.

Experiment 43 (fig. 3). In this experiment the jugular and portal venous pressures were followed in addition to the arterial pressure and the femoral inflow. The dog weighed 5.8 kilo. The injection of 8 cc. of a 1: 1000 solution of adrenalin in the course of 28 minutes at first raised the arterial pressure from 100 to 140 mm. Hg., but the pressure soon fell to 122 and after some 21 minutes began to fall again and at such a rate that, by the completion of the injection, the arterial pressure was back to its initial level, and still was falling. It had fallen to 90 mm. Hg. when, without any apparent cause or warning, respiration stopped, *c*. Ar-

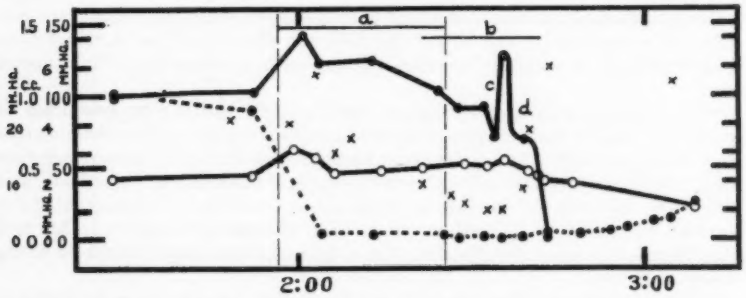

Fig. 3. Experiment 43. Effect of injecting adrenalin. Arterial pressure, —●—●—; femoral inflow, - -●- -●- -; portal pressure, —○—○—; jugular pressure, x x x. *a*, injecting 8.0 cc. adrenalin, 1: 1000; *b*, heart irregular; *c*, respiration stopped, artificial respiration started; *d*, heart stopped.

tificial respiration, without ether, was immediately inaugurated. The arterial pressure abruptly fell 20 mm. Hg. but rose again, presumably under the influence of the artificial respiration and possibly also of a change in the character of the heart beat, to 127 mm. Hg. This pressure was maintained only as long as the changed heart beat lasted,—about 2 minutes; then the arterial pressure fell back to, indeed, somewhat below the level from which the sudden rise had started. Shortly thereafter, however, the heart very suddenly and unexpectedly stopped beating. We are of the opinion that in this case the sudden cessation of respiration and also the sudden stoppage of the heart were due to the direct and separate action of the adrenalin upon the medullary center and upon the heart. Whether this action was exerted directly upon the organs in question or indirectly through their blood vessels is a question that must be left open. This statement is made in full knowledge of the almost universal belief that adrenalin has but little action upon the blood vessels of these organs. We will have occasion to refer to this topic again.

The femoral inflow was practically stopped by the injection. It did not increase materially until the animal died, and it was still far below normal even 25 minutes after death. The fact that the inflow accelerated perceptibly at the moment the animal died may be taken to mean that during life the peripheral action of the adrenalin had been reinforced somewhat through central action. It is obvious, however, that in experiments of this character it matters little what the center may be doing,—for the vessels are maximally constricted by the direct action of the adrenalin.

The injection raised the portal pressure promptly from 10.5 to 15.5 mm. Hg., but in the course of 9 minutes it dropped back practically to the original level. It then slowly rose to 13 mm. Hg., fluctuated slightly at the time respiration stopped, and then fell after death, though no more rapidly than before.

Although these observations on the portal pressure cannot be interpreted in the absence of information with regard to the simultaneous behavior of the splanchnic, at least, if not also of the hepatic, resistance, they are none the less of some value in that they indicate that a high portal pressure is not the cause of the circulatory disturbance. The height to which the portal pressure is raised here is not sufficient, presumably, to cause a dangerous retention of blood in the portal area.

The jugular pressure, excepting one reading made shortly after beginning to give the adrenalin, falls progressively during and subsequently to the administration of the adrenalin. It mounts again, and quite rapidly, at the time respiration fails. This was probably caused by the pressure of the artificial respiration, and in part by the failure of the heart. It should be noted that the inception of the cardiac irregularity, *a*, is without effect upon the jugular pressure. This fact may be taken to mean that the arrhythmia did not reduce the effectiveness of the heart as a pump.

In this experiment, therefore, the fall in arterial pressure occurring between the termination of the injection and failure of the heart cannot be attributed to loss in arterial tone, for the arteries are markedly constricted (unless other areas do not behave as does the femoral area, and of this more later); and probably not to failure of the heart, for then the jugular pressure would have risen, as it did when the heart actually failed at the end; nor to accumulation of blood in the portal area, for the portal pressure is not high enough to hold out of the general circulation enough blood to dangerously reduce the volume of blood in circulation; nor to the accumulation of blood in the systemic veins, for the jugular pressure is not increased. We are then forced to ascribe the fall in arterial pressure to a reduction in the volume of blood returned to the heart.

Experiment 44 (fig. 4) gives a more prolonged picture of the after-effects of adrenalin injection. The same observations were made as in the preceding experiment. The injection of about 8 cc. of 1: 1000 adrenalin in the course of 21 minutes into a dog weighing 7.5 kilo, but exceedingly fat, raised the arterial pressure from 84–95 to 154 mm. Hg. If we neglect some variations that seem to be of no present significance, the arterial pressure then fell with a constantly diminishing rate until the end of the experiment approached. The animal died presumably through eventual, though gradual, failure of the respiration. With the failure of respiration and the consequent failure of the heart, the arterial pressure of course, fell more rapidly again.

The femoral inflow was practically stopped by the adrenalin. The peripheral vessels began to dilate appreciably only some 40 to 50 minutes after terminating the injection, and only an hour before respiration failed. At the moment of death the inflow rate was still far below the initial level. In this experiment, again, we see the effect of final failure of the center upon the tone of the peripheral vessels; the dilatation occurring at this time proves that the constriction is only partly due to the peripheral action of the adrenalin. The low arterial pressure presumably so stimulates the center as to cause it to maintain a higher grade of constriction than the peripheral action of the adrenalin alone is capable of maintaining. It can also be seen that the arteries are not completely dilated even 1 hour 20 minutes after death. In large part this slow port-mortem inflow rate is due to something transpiring in the main artery; for the inflow rate into the unclipped artery is only a bit faster than the inflow rate into the clipped artery. The first impression one gains upon encountering this phenomenon is that a clot has formed in the main artery. But this can not be the case, for with death there is always

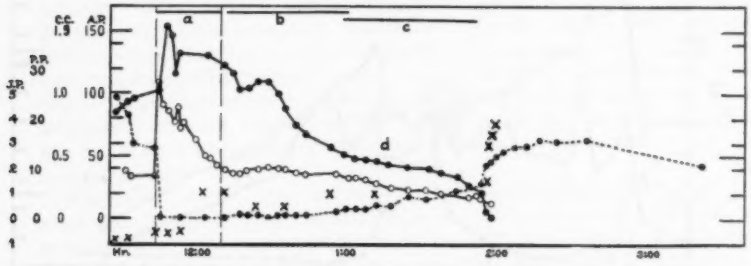

Fig. 4. Experiment 44. Effect of injecting adrenalin. Arterial pressure, —●—●—; portal pressure, —○—○—; femoral inflow, - -●- -●- -; jugular pressure, x x x. *a*, injecting 8.0 cc. adrenalin, 1:1000; *b*, heart very irregular; *c*, respiration diminishing.

an acceleration similar to the one here seen. This would be impossible if the slowing were due to the presence of a clot.

The adrenalin promptly raises the portal pressure from 8.0 to 9.5 to the astounding height of 27.0 mm. Hg.; but the pressure immediately begins to fall and by the end of the injection period it has returned within the initial range where, to all intents and purposes, it remains for about an hour. But finally, and despite the gradual dilatation of the leg (and also, presumably, of the intestinal) arteries, the portal pressure falls well below its initial level.

The jugular pressure rises during the injection period from about -0.5 to 1.0 mm. Hg. where it remains with inconsequential fluctuations until the heart practically ceases to beat, when it rises to 3.5 mm. Hg. There is no obvious change in venous pressure in association with the beginning and the termination of the period of cardiac irregularity such as might be expected if the irregularity were associated with a change in the efficiency of the heart.

In this experiment, to summarize, the arterial pressure falls despite a strong peripheral constriction and despite a portal pressure, which, excepting only the

period of injection is normal or below normal. To the eye the mesenteric veins, in this case, seemed to be small. There is a slight rise in jugular pressure as a result of the adrenalin injection. This might be considered as the expression of an accumulation of blood in the systemic veins, due either to diminution in the efficiency of the heart or to a diminution in the capacity of the arteries. The heart was irregular for a time, but the record is without evidence that this irregularity interfered with the work of the heart. An insufficient supply of blood to the heart seems, therefore, to be the only factor remaining to account for the failure of the circulation. This animal was definitely in shock during the last 50 minutes of the experiment.

Experiment 46 (fig. 5). In this experiment the intestinal inflow was followed instead of the femoral inflow. The dog weighed 7.9 kilo. The first dose of adrenalin was 9 cc. of 1 : 1000 in the course of 28 minutes. It raised the arterial pres-

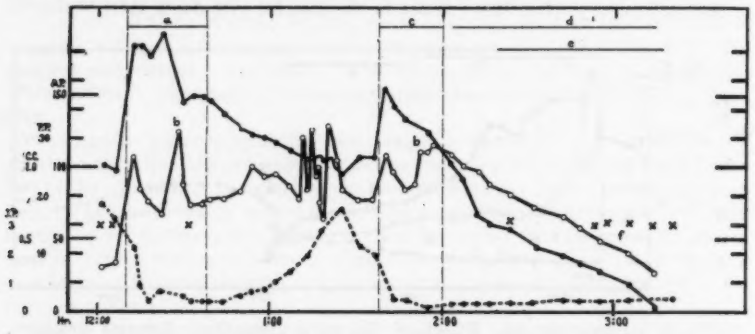

Fig. 5. Experiment 46. Effect of injecting adrenalin. Arterial pressure, —●—●—; portal pressure, —○—○—; intestinal inflow, - -●- -●- -; jugular pressure, x x x. *a*, injecting 9.0 cc. adrenalin, 1: 1000; *b*, brief pause to refill syringe; *c*, injecting 11.0 cc. adrenalin, 1: 1000; *d*, heart irregular; *e*, respiration slowing; *f*, starting artificial respiration.

sure from 100 to 180, indeed, to 190 mm. Hg. Before the completion of the injection the arterial pressure began to fall and, barring certain fluctuations which do not concern us here, it declined gradually to its initial level, when the second dose was administered.

The intesinal inflow rate was markedly decreased during the injection, but it did not fall quite to zero. It increased subsequently and became normal in the course of 47 minutes. But then, corresponding with a definite rise in the arterial pressure, the peripheral resistance again increased.

The portal pressure was markedly increased by the injection and it remained high, though irregular, subsequently. The jugular pressure did not fluctuate more than 0.5 mm. Hg. during the entire course of the experiment, not even when the heart was irregular and the respiratory rate slow.

At 1:10 a series of interesting, though perhaps irrelevant waves in arterial and portal pressure was registered. In the original record it can be seen that the

pressure in the splenic (portal) vein rises very abruptly at the instant the arterial pressure begins to fall. It is to be presumed, therefore, that the cause of this phase of each wave is a splanchnic dilatation.

The circulatory conditions were not quite normal when the second injection of adrenalin was begun. The arterial pressure, to be sure, was about normal, but this normal level was maintained apparently through over-activity of the vasomotor center; and the portal pressure (18 mm. Hg.) was considerably above the initial level. Inasmuch as the intestinal arterioles were somewhat constricted at this time, it follows that the high portal pressure must be attributed to a continued high portal resistance. The conditions with respect to the portal pressure following the first injection evidently were very different from those heretofore observed: they were favorable to the retention of blood in the abdominal viscera. Yet the circulation showed no definite evidences of failing.

The second injection of adrenalin (11 cc. of 1: 1000) now raised the arterial pressure to 152 mm. Hg.: but then, despite the continuance of the injection, the pressure steadily fell and the heart stopped about 1½ hours after terminating the injection. The intestinal inflow dropped practically to zero and showed very little tendency to increase even after the animal died. By the test described in another place (3) it was found, shortly after the completion of the second injection, that the slowing of the inflow rate was due to constriction of the main inflow artery. The portal pressure rose irregularly during the injection period and then fell steadily, crossing the pre-injection level after about 20 minutes had elapsed. The fall in the portal pressure undoubtedly was due to the coincident fall in the arterial pressure.

The possibility that the fall in the arterial pressure in experiments of this character is due to dilatation in the splanchnic area while the somatic (femoral) area is constricting, finds no support in the results of this experiment. Furthermore, the failure of the jugular pressure to change materially seems to exclude an accumulation of blood in the systemic veins through functional incapacity of the heart as a factor in the failure of the circulation. This is the first experiment in which the portal pressure has remained at a high level during most of the injection and post-injection periods. Therefore, in this case the possibility that the failure of the circulation is due to accumulation of blood in the portal area cannot be excluded, though, as has been said, the portal pressure after the first injection remained high and yet the circulation was satisfactorily maintained. It is recorded in the notes that the mesenteric veins were distended.

Experiment 48 (*fig. 6*). The intestinal inflow was followed in this experiment also. The animal weighed not over 7 kilo and received a large dose of 1: 1000 adrenalin (about 11 cc. in the course of 21 minutes). The arterial pressure was elevated at once from 100 to 170 mm. Hg.; it then immediately began to fall. With the exception of two irregularities this fall, upon the whole, was steady, and in the course of 2½ hours carried the arterial pressure down to 80 mm. Hg. Of the two irregularities in the pressure fall, the first was a fall and rise in association with a very decided temporary slowing of the respiration, *c*. We believe these two events were causally related and are indicative of some transitory central change. It will be noted that at this time, judging by the inflow rate, the adrenalin action was at it height. The second change consisted of an abrupt rise in the arterial pressure which undoubtedly is to be attributed to the disappearance of the heart

irregularity, *b*, which had persisted almost from the very beginning of the injection.

The inflow rate decreased during the injection, reaching zero with the first estimation after the cessation of the injection. It then gradually increased, but even 2 hours later it was still decidedly below normal. The portal pressure at first rose from 5 to 18 mm. Hg., fell somewhat, and then rose to attain a second crest about 70 minutes after beginning the injection. It then fell more or less steadily. The form of this part of the portal curve obviously is influenced by the arterial pressure. The fall in arterial pressure is not, however, the sole cause of the fall in portal pressure; for while the arterial pressure falls from 107 to 80 mm., a ratio of 1.3 to 1, the portal pressue falls from 16 to 5 mm., a ratio of 3.2 to 1. In other words, the portal pressure falls faster relatively than the arterial pressure, and this more rapid fall in portal pressure occurs despite a progressive dimi-

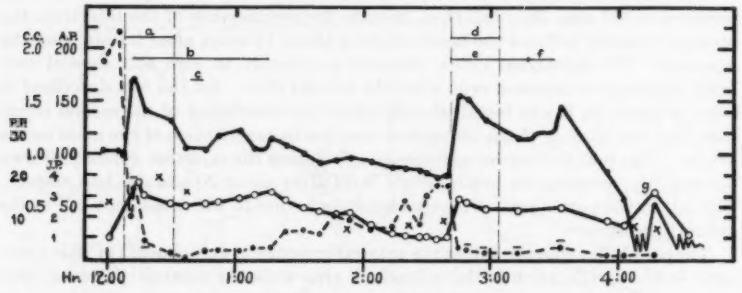

Fig. 6. Experiment 48. Effect of injecting adrenalin. Arterial pressure —●—●—; portal pressure, —○—○—; jugular pressure, x x x; intestinal inflow, - -●- -●-- (for the readings marked by the sign (−) the inflow pressure was on a much higher than the usual level). *a*, injecting 11.0 cc. adrenalin, 1: 1000; *b*, heart irregular; *c*, respiration slow; *d*, injecting 11.0 cc. adrenalin, 1: 1000; *e*, heart irregular; *f*, change in type of heart irregularity; heart regular subsequently; *g*, artificial respiration; the heart at this time was beating periodically.

nution in the splanchnic resistance which ordinarily would tend to raise the portal pressure. To be in a position to interpret these changes in the portal pressure it would be necessary to know also the state of the portal resistance in the liver. Since this information is not at hand a decision with regard to the cause of the more rapid fall in the portal pressure cannot be reached.

The continuous fall in arterial pressure probably is not due to increasing incapacity of the heart. The jugular pressure to be sure is raised somewhat (1 mm.) during the administration of the adrenalin, but later it falls below the initial level; and though the heart beat under the influence of the adrenalin becomes irregular, this irregularity, *b*, does not persist.

A second dose of adrenalin, similar to the first, was now given. At this time the circulation had by no means returned to its initial state. The arterial pressure, despite a relatively high peripheral resistance, was at least 20 mm. Hg. below normal. Whether conditions would in time have become normal is a ques-

tion that cannot be answered. The portal pressure, though, had returned to its initial level. As a result of the injection the arterial pressure rose to 156 mm. Hg. and the heart became irregular. The arterial pressure then fell steadily until, 40 minutes later, a change in the character of the heart irregularity, *f*, apparently caused the pressure to rise slightly; another and greater rise occurred 10 minutes later when the heart beat became regular. The fall in arterial pressure was then resumed, a decrease from the level of 144 to 57 mm. Hg. taking place in the course of 30 minutes. The heart then exhibited a peculiar type of irregularity, the nature of which does not concern us here.

The intestinal inflow rate was cut down by the injection almost to zero, and had not begun to increase even 90 minutes later, when the heart became irregular.

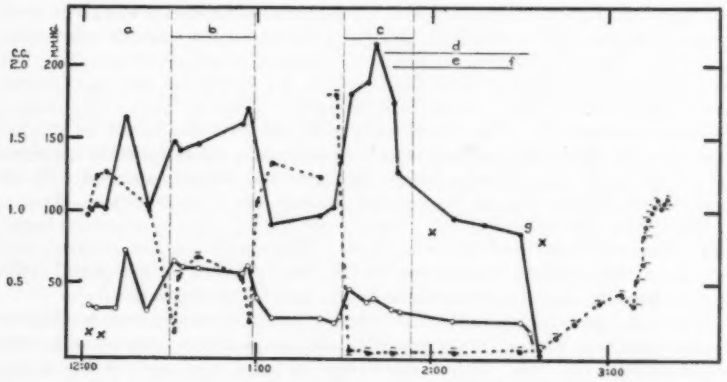

Fig. 7. Experiment 49. Effect of injecting adrenalin, small and large doses. Arterial pressure, —●—●—; portal pressure, —○—○—; jugular pressure, x x x; intestinal inflow, - -●- -●- - (for the readings marked by the sign (—) the inflow pressure was on a much higher than the usual level). *a*, adrenalin injection started and discontinued; *b*, injecting 11.0 cc. adrenalin, 1:10,000; *c*, injecting 11.0 cc. adrenalin, 1:1000; *d*, heart irregular; *e*, respiration markedly slowed, stopped at *f*; *g*, artificial respiration started.

The portal pressure was permanently elevated by the injection; it showed some slight fluctuations which were always of the same sign as the large fluctuations in the arterial pressure. Here, therefore, the arterial pressure falls despite a high peripheral resistance and despite the absence of any evidence of cardiac failure. The portal pressure, however, remained high during most of the experiment. Therefore, the possibility that the fall in arterial pressure is due to the accumulation of blood in the splanchnic veins cannot in this case be excluded.

Experiment 49 (*fig. 7*). This experiment was performed primarily for the purpose of comparing the effects of small with the effects of the large doses of adrenalin given in previous experiments. The first attempt to inject 1:10,000 adrenalin, *a*, had to be discontinued on account of an accident. Then, after the circulation had returned to its previous state, 11 cc. of 1:10,000 adrenalin were given

over a period of 28 minutes, *b*. During the injection period the arterial and portal pressures, as usual, were raised, the intestinal inflow rate slowed. Though this is also the result commonly obtained with the larger doses there are, nevertheless, certain outstanding differences. These are,—the steady rise in arterial pressure, the absence of any tendency on the part of the portal pressure to fall after the preliminary rise, and the incompleteness of the peripheral constriction. In the period subsequent to the injection, the striking differences are,—the immediate relaxation of the arterioles beyond their initial size, with a return to the normal inflow rate in the course of 20 minutes; the immediate descent of the arterial pressure to a subnormal level with a relatively rapid recovery to the normal; and the almost immediate return of the portal pressure to the normal level. The circulation became normal in every respect.

The second injection consisted of 11 cc. of 1: 1000 adrenalin; it was given in the course of 24 minutes, *c;* this dose, therefore, was about ten times as large as the first dose. The arterial pressure rose to the unusual height of 216 mm. Hg., but, as usual, began to decline before the injection was completed, and at about the time, *d*, the heart became permanently irregular. Slightly later, *e*, the respirations became very slow and this slowing increased until the breathing stopped altogether, *f*. The heart stopped after the respiration, and despite the inauguration of artificial respiration, *g*, before the heart had actually stopped. By the time the respiration stopped the arterial pressure had fallen to 84 mm. Hg.

The inflow rate fell practically to zero when the injection of adrenalin began, aud it remained there until the animal died. Then some relaxation occurred, indicating that the previous constriction, though mostly due to the peripheral action of the adrenalin, had also been maintained in part by central action.

The portal pressure was instantly increased by the adrenalin but immediately began to decline, while showing some fluctuations in correspondence with those of the arterial pressure. It was subnormal during a large part of the period. The jugular pressure was markedly increased; for this it is possible that both the altered respiration and heart action were responsible.

It can scarcely be maintained that this animal was brought into a state of shock; but it is of significance that at the end the arterial pressure was somewhat subnormal despite an intense arterial constriction. Death was due to some action of the adrenalin, direct or indirect, upon the respiratory center.

Summary and discussion of experimental data

Now, with a variety of results before us, an attempt will be made to ascertain the usual reactions to the injection of adrenalin and the significance of those reactions in relation to the shock problem.

1. Action on the arterial pressure. The arterial pressure *during the injection* period invariably is high, and higher during a first than during a second injection. When the dose is relatively small the arterial pressure usually is consistently high (figs. 2, *a* and 7, *b*); but when the doses are large the pressure usually begins to fall before the end of this period (figs. 4, *a*; 5, *a*; 6, *a*). One gains the impression that the larger the dose

the earlier and the more profound is this fall in pressure apt to be. It always is more marked after a second than after the first dose. The decline during the injection period, though, usually falls short of carrying the arterial pressure back to its initial level. These differences in the effect of dosage on the arterial pressure may depend in part upon differences in the action of the adrenalin upon the heart; but that the fall in pressure is not wholly dependent upon the behavior of the heart is indicated by the fact that it may begin before the irregularity (fig. 4) and may not occur when the heart becomes irregular (fig. 2, *b*). A second large dose never raises the arterial pressure as high as did the first dose, provided it also was large.

After the administration of a small (fig. 7, *b*) or of a moderate dose (fig. 1, *a*) the arterial pressure usually falls rather abruptly to a subinitial level, and then mounts slowly, eventually reaching normal, after small doses (fig. 7, *b*), but usually not regaining the normal level after moderate doses (figs. 1, *a*; 2, *a*). A second dose that produces approximately the same immediate reaction, leaves the arterial pressure still lower (fig. 1, *c*). After a large first dose, barring accidental occurrences, the fall in arterial pressure begun during the injection period continues without any break to mark the conclusion of the injection (figs. 4, 5, 6), it may be, until the animal dies (fig. 4); though this is more commonly seen after a second large dose (figs. 2, 5, 6). A decline of this character that does not kill, tends to terminate below the initial level (figs. 5, *a*; 6, *a*). So far as can be judged by the arterial pressure, therefore, large doses of adrenalin do long-lasting damage to the circulation, if they do not actually carry the pressure down to a dangerously low level.

2. Action on the peripheral resistance. Without exception the response to adrenalin during the injection period has been a marked peripheral constriction causing, in the case of the larger doses, practically a complete cessation of inflow (figs. 2, 3, 4, 5, 6, 7).

After terminating the injection of a small dose (fig. 7, *b*) the resistance quite promptly decreases to, or slightly below, normal; after a moderate dose (figs. 1, *b*; 2, *a*) the relaxation that occurs is apt to be slower and short of complete return to normal; while after large doses, and often after moderate doses given a second time, the relaxation is very slow and usually very far from complete even after two hours or more have elapsed (figs. 4; 5, *c*; 6, *d*). This long-lasting constriction, however, usually is not wholly due to direct action of the adrenalin upon the blood vessels; a part of the tone is central in origin as is evidenced by the acceleration of dilatation that usually occurs at the moment

the animal dies (figs. 3, 4, 7). The splanchnic and the femoral areas are alike in their response to these doses of adrenalin. In one experiment (fig. 1, *a*) the constriction of the period of injection gave way very rapidly to an extreme dilatation, followed at once by a gradually developing constriction which probably was central (compensatory) in origin.

The literature on the action of adrenalin on the blood vessels has recently been reviewed by Hartman (4). There the statement occurs that "dilatation in the blood vessels of skeletal muscles is the usual response to adrenalin in animals." Dilatation is also said to occur in the intestinal vessels, especially after large doses (5), (6). Our findings do not agree with these statements. To be sure, the method we have employed in following the peripheral resistance furnishes discontinuous information; and if very brief periods of dilatation occur during the continuous injection of adrenalin there is the possibility that our method might fail to detect them. The fact, however, that we have never encountered dilatation *during* the injection of adrenalin certainly renders this possibility a very remote one, indeed. In the post-injection period, as has been said, we have encountered striking and momentary dilatation, but only once (fig. 1, *a*); and a very slight transitory dilatation, also, only once (fig. 7, *b*). With these exceptions, the constriction produced by both moderate and large doses has always outlasted the injection period, in some of the instances as long as 2 hours.

Hoskins, Gunning and Berry (7) found that adrenalin in "all" doses constricts the skin vessels but not those of the muscle. Later, however, Gunning found (8) that massive doses of adrenalin slow the blood flow through muscle. This correction suggests that all of the discrepancies depend merely upon dosage and method of administration. However this may be, it is certain that the constriction we have observed is so marked that it cannot, in the case of the femoral readings, be accounted for on the basis of a constriction affecting the skin vessels alone.

Gruber (9) fails to obtain dilatation of the blood vessels of muscle shortly after section of the nerve; neither, apparently, does he, under such circumstances, obtain constriction. Even, therefore, if it were admitted for purposes of argument that we get constriction because the nerves have been injured (and such experiments as 41 [fig. 1, 2nd injection] conclusively show that constriction is obtained when the nerves unquestionably are active), the reduction in inflow rate that we obtain is far too great to be referable to constriction of the skin vessels alone.

But whatever the cause of the discrepancy, we can assert *a*, that our method of determining the effect of the adrenalin upon the peripheral resistance is free of the ambiguities that attach to the outflow and plethysmograph methods employed by other investigators; *b*, that our method demonstrates that in the dosages we have employed constriction of both the somatic and the splanchnic areas is the main if not the only effect of the continuous injection of adrenalin; and *c*, that this constriction often persists for hours after the dose has been administered, partly, perhaps, as a central compensatory response to the low arterial pressure, or to a tendency to a low arterial pressure.

Our method of following the vasomotor tone does not help to dissociate the factors, central and peripheral, upon which the constriction depends. There is, of course, no doubt but that during the injection of the adrenalin any part the center may be playing in maintaining the constriction must be insignificant in comparison with that effected by local action. That the center, however, is a factor in maintaining the constriction subsequent to the injection is indicated by the acceleration of the recession of the constriction that often occurs at the moment the animal dies. But even with death the dilatation is relatively slight. This failure of the injection rate to return to, and then to pass beyond, the initial value probably is a manifestation of the phenomenon that has already been described as occurring at the termination of long experiments in which no adrenalin whatever has been employed (3), and which we felt might be related to the phenomenon of post-mortem contraction described by MacWilliam (10). In the present experiments, however, it is possible that the long-lasting constriction is due to the direct action of the adrenalin upon the walls of the large arteries (11).

3. The systemic venous pressure. Our method of measuring the venous pressure gives, to all intents and purposes, the pressure in the right auricle. Pressure changes here are of course slight at best, and are affected not alone by the force from behind and by the capacity of the heart to empty itself, but also by variations in the effectiveness of the respiratory pump. It is not surprising, therefore, that the literature on this subject is contradictory. Thus Bainbridge and Trevan (12), for example, find that the pressure in the vena cava is not altered by the injection of adrenalin; whereas Schmid (13), Elliott (11) and Edmunds (15) say it is elevated.

Our results in this respect have been quite inconstant. In one experiment (fig. 5), the jugular pressure changed but little; in two (figs. 3, 6) it fell; and in two instances (figs. 4, 7) it rose. In both of the last,

though, there were two possible and perhaps unusual causes for the rise. One cause was a change in the character of the respirations: in one case (fig. 4) this change was slight and the venous pressure was elevated but little; in the other (fig. 7), the respiratory change was very marked and the rise in venous pressure also was marked. The other possible cause of the rise in venous pressure is related to the effect the adrenalin had on the portal pressure. For these two experiments were exceptional in that after the injection the portal pressure (see below) quickly resumed its normal level. The marked constriction of the arterioles caused by the adrenalin and the relative emptiness of the portal veins that a normal pressure in them bespeaks, together might have had the effect of distending the systemic veins. But whatever the cause of the inconstancy of the jugular pressure may be, it is obvious that it permits us to conclude only that the heart as a rule is capable of moving on the blood that is brought to it.

Action on the heart. In many of the experiments there were indications of deleterious action of the adrenalin upon the heart, the heart developing a very marked irregularity either while the adrenalin was being injected or subsequently. This irregularity was not of the kind that is produced by vagal action. The arterial pressure at these times occasionally (figs. 1, *d, e;* and 6, *b*) gave evidence that the heart was not as efficient as when it was beating regularly; at other times, however, the inception or the disappearance of the irregularity was without obvious effect upon the arterial pressure. Furthermore, with but one possible exception (fig. 7, 2nd dose), the jugular pressure failed to exhibit with the inception and disappearance of this irregularity, the alterations that are taken to indicate diminishing or increasing capacity of the heart for work. No effort has been made to ascertain the nature of the irregularity. But whatever it may be, it seems justifiable to conclude that failure of the heart is not an important factor in the *gradual* failure of the circulation after the administration of adrenalin. The *sudden* stoppage of the heart that occasionally has occurred while the arterial pressure still was sufficient (figs. 3 and 7) may have been caused by some direct action of the adrenalin upon the heart. But this is a question that does not concern us here.

Action on respiration. The adrenalin in almost every instance has had a marked effect upon the respiration. This has consisted *a*, of transitory slowing (fig. 6, *c*); *b*, of progressive slowing of the respiratory rate (figs. 4, *c;* 5, *e*), sometimes leading to surprisingly low rates (6 per minute in fig. 7); or *c*, of stoppage of respiration, sometimes sudden (fig.

3, *c*), sometimes preceded by slowing (fig. 5, *f*). The administration of artificial respiration in the latter instances at most has delayed death only a few minutes (figs. 3, 5). Therefore, lack of pulmonary ventilation can scarcely be regarded as the cause of death or of the breakdown of the circulation. It is not the purpose of this paper to discuss the cause of failure of the respiratory center.

The portal circulation. Except, perhaps, its action on the peripheral resistance, the most interesting effects of adrenalin are those it exerts upon the portal circulation. The first effect of the injection of adrenalin invariably is to raise the portal pressure, it may be as high as 28 (fig. 5) or 30 (fig. 6) mm. Hg., though usually to not over 15 mm. Hg. The portal pressure then usually falls practically to the initial level *a*, either during the injection (figs. 3 and 7, 2nd dose), or *b*, immediately (figs. 4 and 7, 1st dose) or some time after (fig. 6); and *c*, while the arterial pressure is still well maintained (fig. 6, 1st dose) or *d*, not until the arterial pressure falls to quite a low level (fig. 5, 2nd dose). In all of these cases there is a certain, although by no means complete, parallelism between the portal pressure and the arterial pressure. In one case (fig. 5, 1st dose) the portal pressure remained high even after the arterial pressure had returned to the initial level.

The very marked effects that these doses of adrenalin have upon the portal circulation suggest the advisability of carefully examining them in order to determine whether they are of significance in relation to the failure of the circulation. Physiologically, a continuous elevation of the portal pressure can be effected *a*, through a rise in arterial pressure; *b*, through a diminution in arterial resistance in the portal area; *c*, through a rise in caval pressure; *d*, through contraction of the musculature of the portal veins themselves; and *e*, through an increase in the resistance to flow through the liver.

a and b. The injection of adrenalin, of course, raises the arterial pressure but at the same time markedly increases the resistance in the arterioles. Whether or not the blood flow into the portal area would increase, therefore, would depend upon the balance that is struck between these two effects. Our inflow determinations show conclusively that the constriction, in the intestinal area, at least, is so extreme that despite the rise in arterial pressure the adrenalin must restrict the blood flow. It is, of course, conceivable that the other pathways into the portal system are not constricted by the adrenalin to the same extent as is the intestinal pathway and that it is through the former that the portal pressure is raised. We have no direct observations bearing on this possibility.

The measurements of the blood flow in the portal veins as effected by adrenalin that are recorded in the literature help to settle this question, though the effects of brief injections only have been studied. With large doses Schmid (13) invariably obtained a marked reduction in blood flow in the portal vein; in one of his instances the blood flow stopped completely for a few seconds. On the other hand, Burton-Opitz (14) finds an increase in portal flow during the phase of high arterial pressure. This increase, though, was never very striking, and evidently very inconstant, as may be indicated by the following values taken from his published data:

EXPERIMENT NO.	BLOOD FLOW, CUBIC CENTIMETERS PER SECOND	
	Before	At crest of arterial pressure
3	4.50	4.54
4	3.05	3.20
5	3.43	3.37
	3.25	2.56

Upon the whole, it seems fair to conclude that the blood flow through the portal area is not increased during the prolonged administration of adrenalin. Granting this, it follows that adrenalin constricts the whole of the splanchnic bed. The rise in portal pressure, therefore, is referable neither to a premised splanchnic dilatation nor to the rise in arterial pressure; though, of course, during the period of constriction, variations in arterial pressure must effect variations of the same sign in the portal pressure.

c. It has already been pointed out that the systemic venous pressure ordinarily is not increased by the prolonged injection of adrenalin; therefore we must seek elsewhere for the cause of the high portal pressure.

d. Edmunds (15) has observed that strips of the portal vein contract when immersed in a solution of adrenalin. Occasionally we have observed during the height of the adrenalin action, a sausage-like appearance of the branches of the portal vein, rings of constriction so complete as to quite obliterate the lumen and to offer an appreciable resistance to the passage of the blood, alternating with segments filled with blood. If this were constant in occurrence it might help to explain the high pressure recorded in the splenic vein; but in many instances these evidences of contraction of the veins are absent.

e. If constriction of the large portal radicles is not the cause of the high portal pressure there remains as the only possible explanation of the condition an increase in the resistance to the passage of blood through the liver. This question has recently been discussed by Bainbridge and Trevan (12). The possibilities in the case are: *a*, constriction of the minute portal radicles in the liver; *b*, constriction of the hepatic or sublobular veins; and *c*, narrowing of the hepatic capillaries. Bainbridge and Trevan are disposed "to regard narrowing of the capillary channels by swelling of the liver cells as the most probable explanation of the obstruction produced by adrenalin to the blood flow through the liver." The evidence they offer in favor of this view is that adrenalin causes the liver volume to increase, while histological examination of clamped-off pieces of liver shows the interlobular and intralobular veins to be engorged with blood.

Our own experiments throw relatively little light on the condition of the blood vessels of the liver. We have not examined the liver during the height of the adrenalin action. The gross appearance presented by the liver at the close of experiments has been rather variable. Occasionally all of the marks of engorgement are evident; more often its edges have been sharp, though in such instances the parenchyma on section often seemed to be bloody. In one case we weighed the liver and found it to be at the lower limit of the normal range. Microscopical examination has been made in only one case and that was one in which the liver at autopsy was found to be engorged. In this instance the hepatic capillaries, upon the whole, were dilated and contained an excess of blood. The corpuscles did not seem to be so closely packed as they are in the intestinal vessels of shocked animals. The liver cells were not swollen.

It is inconceivable, indeed, that the liver cells could swell so rapidly under the influence of adrenalin as to cause the practically instantaneous increase in liver volume and portal pressure which are pictured by Bainbridge and Trevan, the more so when it is recalled that even a comparatively small dose of adrenalin may cause a marked increase in portal pressure (see fig. 7, *a* and *b*). As a matter of fact, we are not convinced that the method employed by Bainbridge and Trevan for the purpose of following the volume of the liver is entirely satisfactory. For an increase in arterial pressure, by increasing the turgidity of the liver lobes, might, depending upon conditions, increase the pressure upon the balloon between the lobes. However this may be, the complete parallelism of their "volume" and pressure curves to each other

make it incumbent upon them to prove that they are actually recording volume changes. Better methods (15) seem to indicate that in the dog, at least, the liver volume is reduced by small, brief intravenous injections of adrenalin. If it could be shown that the engorgement of the liver that sometimes occurs after the administration of adrenalin is slow in development, another possible cause of the swelling of the liver cells and of the engorgement of the liver capillaries observed by Bainbridge and Trevan would be the effect upon the tissues of the marked slowing of the blood stream that accompanies the prolonged injection of adrenalin. The process would then be similar to the one which in our opinion accounts for the engorgement of the intestinal capillaries.

Assuming, for purposes of discussion, that the portal action of the adrenalin is the cause of the shock-like failure of the circulation, let us try to discover the mechanisms that might bring it about.

1. When the portal pressure is high, such large amounts of blood might be trapped in the portal area as to lead to serious depletion of the systemic vessels. It is here that the exact location of the increased resistance in the liver might be of some significance. For if the obstruction were beyond the capillaries a very much larger volume of blood would be trapped in the portal area than if the obstruction were in the portal venules. Bainbridge and Trevan (12) have suggested that the distention of the liver and portal area might be a contributory factor. But in certain cases, at least, the trapping of blood cannot be sufficient to be the sole cause of circulatory failure, for the rise in portal pressure may be transitory even in cases that die (fig. 4). Furthermore, the liver, at least at the time of death, as has been said, often does not contain more than its normal quota of blood. Nor are the tributary veins of the portal area always distended during the injection; often they seem relatively empty, though the pressure in them may be high. And finally, experiments to be described later show that a mere rise in portal pressure even when maintained for hours does not embarrass the circulation nearly to the same extent as does the administration of adrenalin.

2. A high portal pressure might reduce the blood volume by increasing filtration of plasma. As a matter of fact, Bainbridge and Trevan find that during the prolonged injection of adrenalin the lymph flow through the thoracic duct is increased. There can be no doubt but that the administration of adrenalin increases filtration pressure in the splanchnic capillaries; therefore, a part of the increase in lymph forma-

tion may be accounted for here. But Bainbridge and Trevan, finding that the lymph "becomes slightly more concentrated," conclude that a part of it comes from the liver. This observation they take as further evidence in support of their contention that the portal obstruction is in or beyond the liver capillaries. There is, however, another possible explanation of the increase in concentration of the lymph, and that is that the extreme and prolonged slowing of the blood stream so damages the hepatic capillary walls (as well as those of other organs) that they become more permeable to the plasma. Again, if we admit for the moment that the portal obstruction is in or beyond the portal capillaries, the filtration would be most rapid at the time the portal pressure is highest, which, as a rule, is during the early stages of the injection. The figures of Bainbridge and Trevan show, however, that the lymph flow steadily increases as long as the injection of adrenalin continues. This observation tends to confirm our view that the part of the lymph that increased concentration indicates is formed in the liver, passes out of the blood stream, not as a result of increased filtration pressure, but rather as a result of increased permeability of the capillary walls.

But after all, those portions of the blood fluids that reach the thoracic duct play no part in the processes that lead to failure of the circulation, for they soon are returned to the blood stream. Only those portions that are retained in the tissues or in the body cavities are of significance in this connection. We have seen no indications of edema of the organs, though during the injection of adrenalin beads of fluid have been seen to form on the surface of the liver. At autopsy the peritoneum occasionally seems to contain a slight excess of fluid.

3. The other striking effect the injection of adrenalin has upon the portal circulation is to slow the blood stream. Here attention should be called to the magnitude of this slowing. The inflow determinations show that even salt solution under a pressure considerably higher than the normal arterial pressure may scarcely flow through either the splanchnic or somatic capillaries during the height of adrenalin action. It is obvious, therefore, that when blood takes the place of salt solution and when two areas of increased resistance (splanchnic and portal) replace the one the inflow has to contend with there can be but little blood flowing through the splanchnic (or the somatic) area.

There are, therefore, good reasons, for believing that failure of the circulation after the administration of adrenalin is the consequence, mainly, of changes wrought in the tissues by asphyxia. In favor of this view is the fact that death has occurred whenever the constriction

has been extreme and long-lasting, and that recovery has occurred whenever the dose of adrenalin failed to maintain a high grade of constriction. Furthermore, the slowing of the circulation in our cases has lasted sufficiently long to lead to failure of the circulation through the same mechanism which, we believe, operates to cause the circulation to fail when the vena cava or the aorta is partially occluded (16). There is, therefore, no need of going outside of this mechanism to find an explanation of the failure of the circulation that follows the long-continued administration of adrenalin, though, of course, such trapping of blood and such filtration of plasma as may occur will contribute to that end.

Pathology. This conclusion is supported by the autopsy findings, for the most striking lesion found is the same marked dilatation of the capillaries and venules of the villi of the intestines that was found by us in shock as produced by exposure of the abdominal viscera, and by partial occlusion of the vena cava or of the aorta. To be sure, the circulatory disturbance resulting from the injection of adrenalin is just the one that would tend to produce this picture, for the high pressure developed in the portal area, at least early in the period of injection, must have the effect of increasing the pressure in the splanchnic capillaries, notwithstanding the sharp fall in pressure that must occur in the constricted arterioles. But that this probably is not the mechanism by which the dilatation is effected, but rather the slowing of the blood flow, is the conclusion that is justified by the results described in the preceding paper of this series (16). In the gross, as well as microscopically, the intestines, stomach and spleen present very much the same appearance as they do in shock as induced by the other methods we have employed.

Does adrenalin produce shock?

Having described and discussed certain of the manifestations of large and long-continued doses of adrenalin the question now arises—are there any reasons for regarding as shock the condition thus induced? If we exclude those cases that die suddenly as a result of stoppage of the respiration or of the heart, we think it can be asserted that at least the type of circulatory failure that is illustrated by experiment 44 (fig. 4), of which we have several instances, in which the pressure gradually falls until the animal dies, bears a close resemblance to the failure of the circulation resulting, for instance, from exposure of the intestines. The fact, furthermore, that animals in this condition may not require an anesthetic, although the eye reflex is still present, if this sign is of any sig-

nificance, lends additional support to the position first taken by Bainbridge and Trevan that shock can be induced by adrenalin. We do not agree with Henderson and collaborators in their conclusion (17) that adrenalin causes death *only* through acute cardiac dilatation or pulmonary edema, though it must be admitted that such accidents (at least the former) often bring the experiment to a close before shock-like failure of the circulation has had a chance to develop.

THE EFFECTS OF OBSTRUCTING THE PORTAL RADICLES IN THE LIVER

Accumulation of blood in the portal area forms the basis of a number of the hypotheses of shock. It is in this way that shock is supposed to result from the splanchnic dilatation of vasomotor paralysis or inhibition; from temporary partial occlusion of the inferior vena cava (18); from constriction or obstruction of the portal hepatic capillaries (12); and from constriction of the latter region and also of the splanchnic capillaries (19). In many of the experiments we have described, a rise in portal pressure, slight and transitory (cava occlusion) or marked and sometimes long-lasting (adrenalin injection), has been a possible factor in the failure of the circulation.

In an effort to reproduce as closely as possible the conditions premised by the hypotheses that attribute shock to an increase in portal resistance, we have had recourse to a method of obstructing the flow of blood through the smaller vessels that has long been practised in physiology, namely, by injecting into the stream of blood flowing toward an organ a suspension of lycopodium spores. These spores have a rough surface and about three to four times the diameter of the red blood corpuscles, and plug the vessels proximal to the point where they break up into capillaries. The increased resistance is thus placed in the situation in which constriction normally occurs, though, if Bainbridge and Trevan are right in their view (and we do not, as has been seen, regard their evidence as conclusive) that the obstruction occurs in the capillaries of the liver, the amount of blood that the injected spores trap in the portal area will not be quite so great as would be held by complete closure of the capillaries.

Method

The suspension of lycopodium spores has been injected into the gastrolienic vein in both the dog and the rabbit. The carotid pressure has been followed in all experiments, and in some of the experiments on the dog the femoral or intestinal inflow rate, the portal pressure and the jugular pressure, also.

Experimental

We will describe and discuss our experiments not in the order in which they were done, nor as entities, but in the way that facilitates the development of the subject.

In *experiment 53 (fig. 8)*, the first, and a relatively small, dose of lycopodium, *a*, raised the portal pressure from the normal of 8 mm. Hg. to a plateau which, in the course of 70 minutes, declined from 12 to 11 mm. Hg. The vasomotor tone was immediately increased and the increase continued for about 25 minutes when,

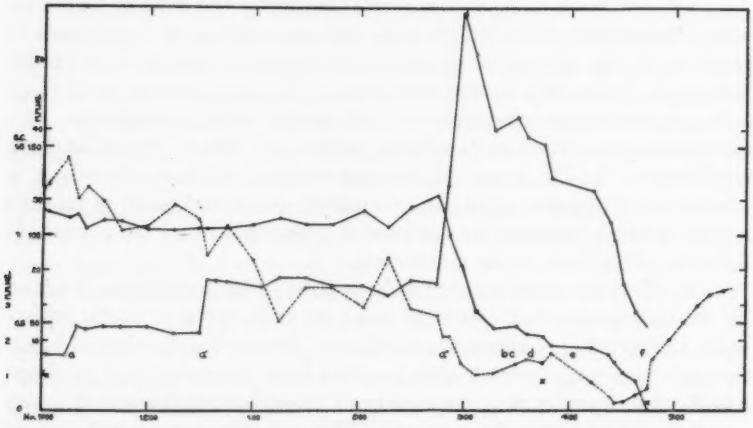

Fig. 8. Experiment 53. Circulatory effects of injecting lycopodium spores into the portal vein. Dog. Arterial pressure, —●—●—; femoral inflow, - -●- -●- -; portal pressure, —○—○—; jugular pressure, x x x. *a*,*a'*, *a''*, lycopodium injected; *b*, accidental injection of carbonate; *c*, artificial respiration; *d*, artificial respiration stopped; *e*, inflow readings transferred to other femoral; *f*, heart stopped.

upon the whole, it decreased slightly but did not return to the normal level. The arterial pressure was affected but slightly, falling from 110 to 102 mm. Hg. Evidently the vasoconstriction sufficed to almost compensate such altered distribution of the blood as was determined by this dose of lycopodium.

Then another dose was administered, *a'*. This raised the portal pressure from 11 to 19 mm. Hg. and, barring certain fluctuations, evidently attributable to passing fluctuations in vasomotor tone, this level was maintained for 2 hours. The inflow rate again was decreased. The general trend of the inflow rate during this period was downward, and of the arterial pressure upward. Evidently at the end of the period vasoconstriction perfectly compensated even this degree of portal obstruction.

We may for the present pass by the last part of this experiment and turn to another experiment (no. 60, fig. 9) in which to all intents and purposes a single, moderately large dose of lycopodium was given. As a matter of fact the lycopodium was given repeatedly in small doses until the desired grade of obstruction was obtained. The immediate effect upon the portal pressure was a tremendous rise from the normal of 6 to 8 mm. Hg., to 41 mm. Hg. But in the course of the next 40 minutes the pressure steadily fell almost to 15 mm. Hg. and, excepting temporary fluctuations, again undoubtedly due to temporary fluctuations in the vasomotor tone, the portal pressure did not vary more than 1 mm. above or below 16 mm. Hg. during the course of the next 3 hours 40 minutes, when the experiment was terminated by stabbing the ventricles *b*. In this experiment the vasomotor tone was followed in the intestines as well as in the leg. We call attention to the fact that the general trend of the tone in these two regions was toward parallelism. The parallelism to be sure was not perfect; but when divergences occurred they were not any larger than one would expect considering that the comparable readings were not made simultaneously. Bearing this in mind, it is seen that

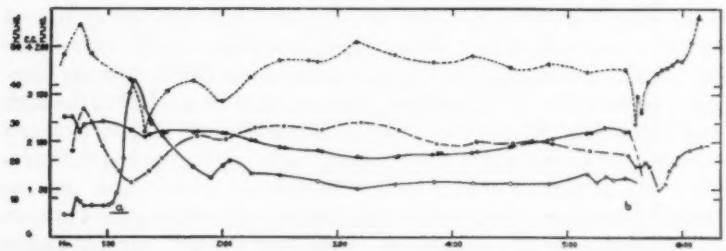

Fig. 9. Experiment 60. Circulatory effects of injecting lycopodium spores into the portal vein. Dog. Arterial pressure, —●—●—; femoral inflow, ··●··●··; intestinal inflow, - - ●- - ●- -; portal pressure, —○—○—. *a*, injecting lycopodium; *b*, heart punctured.

simultaneously with the rise in portal pressure the peripheral resistance increased and the arterial pressure fell, though not more than 15 mm. Hg. During the rapid decline in portal pressure the vasomotor tone diminished but the arterial pressure, after recovering somewhat, remained quite stationary.

We pause here to discuss the mechanism of these occurrences. It is important to ascertain first why the portal pressure steadily falls after the first rise. This fall cannot be attributed to splanchnic constriction because, during this period, the intestinal arterioles are dilating; nor can it be attributed to a change in arterial pressure, for this is rising slightly or is stationary. Neither can it be explained upon the basis of a fall in systemic venous pressure, for in other experiments (no. 53, fig. 8) it was found that this pressure is altered but little by lycopodium injections. An increase in the capacity of the portal area cannot be

invoked as an explanation of the fall in portal pressure because the change in tone of the arterioles and in the arterial pressure indicates that the circulation is improving, which could not be the case if blood were passing out of effective circulation. The only acceptable explanation must provide a gradually widening pathway out of the portal area. Three ways in which this might occur suggest themselves: *a*, The resistance to the passage of blood through the portal venules might diminish as a result of the washing out of the obstructing spores; *b*, the high portal pressure might open up by distention both plugged and unplugged venules. Both of these possibilities are rendered highly improbable by the fact that the same rise and fall in the portal pressure is seen (fig. 11) even when the dose of lycopodium without doubt is large enough to completely occlude the liver capillaries; and by the fact that after any dose large enough to raise the portal pressure above 15 to 20 mm. Hg., the portal pressure subsequently falls and comes to rest somewhere between 15 and 20 mm. Hg. irrespective of the size of the dose. *c*, The foregoing observation, on the other hand, strongly supports another possible explanation, namely, that as a result of the high portal pressure a collateral circulation is established, by way of which the portal blood can reach the systemic veins with ever increasing facility. The pathways that might be developed under these circumstances are familiar to the anatomist; they are the ones opened in man by any chronic portal obstruction. Upon the basis of our experience it can be concluded that with normal arterial pressure and splanchnic resistance, the resistance of the fully opened collaterals is such as to maintain a portal pressure of from 15 to 20 mm. Hg.

Returning now to the analysis of experiment 60 (fig. 9) we believe, on the basis of the foregoing discussion, that during the rapid fall in portal pressure blood is being restored to the general circulation. It is this that accounts for the maintenance of a constant arterial pressure despite a diminishing peripheral resistance. The peripheral resistance continued to diminish until, by about 3.10, it had become quite normal. At this time, though, the arterial pressure was definitely below normal (84 mm. Hg.). It could hardly have been otherwise; for the portal area at this time must have contained more than its normal volume of blood, and the peripheral resistance was normal. During the rest of the experiment, and until the animal was killed, the peripheral resistance and the arterial pressure both slowly increased, the former finally becoming somewhat above normal, the latter, quite normal.

This then represents the extent of the circulatory disturbance that results from holding the portal pressure, for the most part, above 14 mm. Hg. for a period of 4 hours 20 minutes by partially obstructing the portal capillaries. It must be concluded, therefore, that the amount of blood that is withheld from effective

circulation by such distention of the portal area as is caused by a rise of the portal pressure to 14 mm. Hg. is not so marked but that it can be compensated almost perfectly and indefinitely (4½ hours). We further infer that this degree of portal obstruction does not result in nutritional or mechanical disturbances of such a grade as to lead to any considerable reduction in total blood volume.

Even the rabbit, with its very much longer intestinal tract, may withstand perfectly similar grades of portal obstruction. This is clearly shown in experiment 56 (fig. 10). The injection of lycopodium here raised the portal pressure from 8 to 16 mm. Hg. The portal pressure then fell gradually to 12.5 mm. Hg. and then rose again to 13.5 mm. Hg. After a transitory rise, the arterial pressure fell from 74 to 66 mm. Hg., but rose again to 72 as the portal pressure fell, and did not vary very much from that level during the rest of the period.

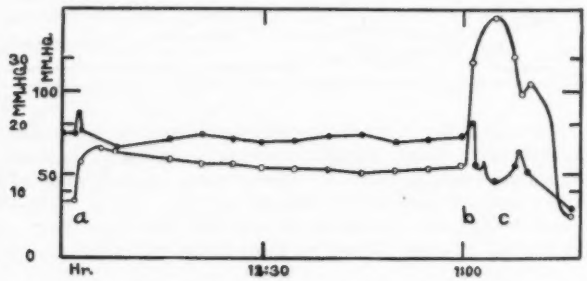

Fig. 10. Experiment 56. Circulatory effects of injecting lycopodium spores into the portal vein. Rabbit. Arterial pressure, —●—●—; portal pressure, —○—○—. *a*, small dose of lycopodium given; *b*, large dose given; *c*, artificial respiration necessary.

The *effect of very large doses of lycopodium spores* may now be considered. This is illustrated by figure 11 (exp. 51). A small dose was given at *a*. As it evidently was not going to be sufficient for our purposes, a very large dose was given at *b*. Considering the effects of the larger dose only, it is seen that the rise in portal pressure again is immediate but that the fall to the plateau is very much more protracted than after smaller doses. There is a profound fall in arterial pressure (to almost 40 mm. Hg.) and a very marked peripheral constriction. The portal circulation is at its worst shortly after 1.00, for the difference between the arterial and portal pressures here amounts to only 10 mm. Hg.; presumably the pressure gradient in the splanchnic area is practically eliminated. But now the circulation improves for a time, presumably as a result of the opening up of byways between the portal area and the systemic veins. Only by invoking some such process is it possible to explain the combination of a fall in portal pressure, a rise in arterial pressure, and an increase in inflow rate. By about 2.20, disregarding a passing fluctuation, conditions have become fairly stable. The arterial pressure is at about 50 mm. Hg., the portal pressure at 15 to 16 mm. Hg., and the inflow rate somewhat below the normal range. The jugular pressure rose throughout the course of the experiment, but the rise amounted to less than 1 mm. Hg. The

picture as a whole can be accounted for perfectly if it is assumed that there has occurred a reduction in the effective blood volume.

Around 3.00 p.m. a change for the worse begins. The arterial pressure falls slowly; the portal pressure rises slowly, and the inflow rate steadily increases. A slow giving way of the vasomotor center, presumably under the influence of the low arterial pressure, satisfactorily explains these changes. At about 4.20 the heart gives evidence of failing, the arterial pressure and the portal pressure both

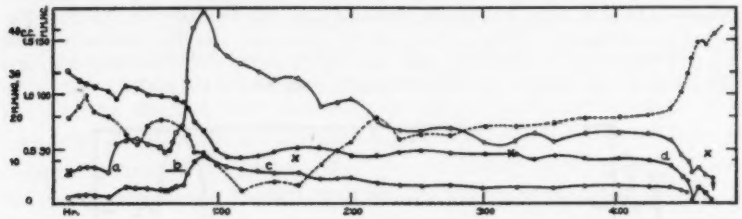

Fig. 11. Experiment 51. Circulatory effects of injecting lycopodium spores into the portal vein. Dog. Arterial pressure, —●—●—; femoral inflow, - -●- -●- -; portal pressure, —○—○— (lower curve on same scale as arterial pressure, upper curve on larger scale); jugular pressure, x x x. *a*, injecting lycopodium, very large dose; *b*, injecting lycopodium, very large dose; *c*, inflow readings transferred to other femoral; *d*, heart becoming irregular.

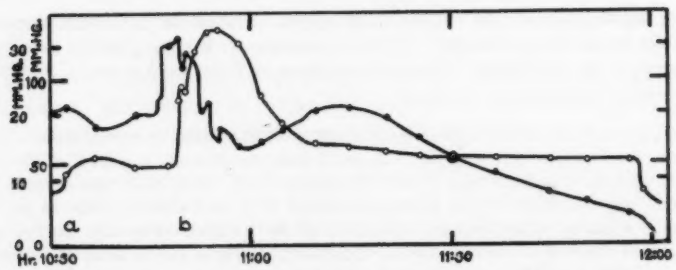

Fig. 12. Experiment 57. Circulatory effects of injecting lycopodium spores into the portal vein. Rabbit. Arterial pressure, —●—●—; portal pressure, —○—○—. *a*, injecting a small dose of lycopodium; *b*, injecting a large dose.

fall rapidly, the vasomotor tone rapidly gives way, and the animal soon dies. It is worth while emphasizing that at no time during the course of this experiment could the ether be dispensed with. Whenever the mask was removed, even after the heart had become irregular (4.20), the animal soon began to struggle.

Figure 8 (exp. 53) shows that a large dose of lycopodium given a dog (at a'') which already has for some hours been under the influence of moderate doses, brings about circulatory failure much more quickly than when it is given at the

beginning of an experiment. The character of the response, though, is alike under both conditions. Thus, in this case we see the tremendous rise in portal pressure followed by a steady and comparatively rapid decline; an immediate sharp fall in arterial pressure followed by a later sharp decline when the level of 50 mm. Hg. is reached; and a further sharp vasoconstriction.

A similar response is obtained from the rabbit when a large dose is given after a small dose has been acting for some time (see fig. 10, exp. 56). But to a larger dose given early in an experiment, the rabbit (fig. 12, exp. 57) seems to succumb much more rapidly than does the dog, though, as nearly as can be judged in the absence of inflow determinations, the response in the rabbit is similar in kind to that given by the dog. As in the case of the dog (fig. 11), we see the rise in portal pressure followed by a fall to a fairly well-maintained level of about 12 to 15 mm. Hg., and a fall in arterial pressure to 50 mm. Hg. followed by a rise which then gives way to a steady fall that leads to death.

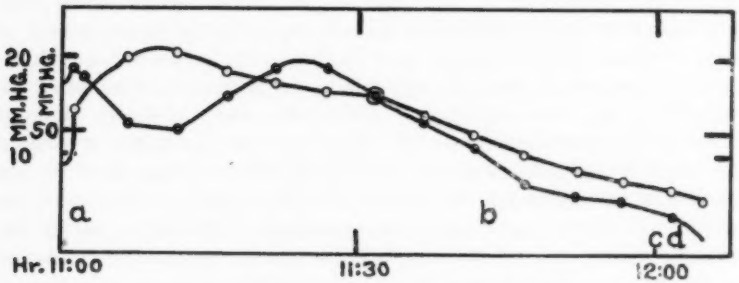

Fig. 13. Experiment 54. Circulatory effects of injecting lycopodium spores into the portal vein. Sick rabbit. Arterial pressure, —●—●—; portal pressure, —○—○—. *a*, moderate dose of lycopodium injected; *b*, respiration shallow, heart irregular; *c*, respiration stopped; *d*, heart stopped.

As might have been anticipated, it was found that the response given by sick rabbits is much more profound than that given by well animals. This is illustrated by figure 13 (exp. 54). The dose of lycopodium suspension given was regarded as moderate. The curve, though, is quite like the one (fig. 12) just described; but it took only an hour for the circulation to fail.

DISCUSSION

These experiments show that an obstruction of the portal radicles in the liver that raises the portal pressure to a much higher level than it is raised by the Janeway and Jackson method of obstructing the vena cava (18), and quite as high as it is raised by the injection of adrenalin, in the dog at least, does not, even after more than 4 hours have elapsed, result in any very considerable alteration in the effectiveness of the cir-

culation as evidenced by the arterial pressure and by the tone of the vasomotor center. A similar statement applies to the results obtained in the rabbit, also. Complete portal embolism in the dog immediately impairs the general circulation, as is evidenced by a low arterial pressure, and eventually (in 3½ hours) causes the vasomotor center to fail. But even in this event the animal does not become apathetic, and therefore does not present the complete picture of shock. In the rabbit, the fatal issue develops more rapidly than in the dog, and the rabbit, if not strong, may rapidly succumb to even a moderate portal obstruction.

Inasmuch as the portal obstructions we have produced by the injection of lycopodium without doubt have been quite as marked as any that could be effected by any physiological or acute pathological process (embolism and thrombosis excepted) we are forced by our results to conclude that acute portal obstruction due to the action of some physiological mechanism, and the consequent accumulation of blood in the splanchnic area, can scarcely be regarded as a cause of shock. Even if it should turn out that the seat of the action of adrenalin is in or beyond the hepatic capillaries, and even if it should be shown that the adrenalin obstructs the collateral, as well as the direct portal circulation, we would still regard our experimental results as sufficient to justify the statement that a portal obstruction *per se* cannot reasonably be regarded as the cause of the failure of the circulation following the injection of adrenalin.

As a matter of fact, we have in the action of adrenalin upon the systemic arterioles all that is necessary to account for the failure of the circulation, namely, the consequences of an extreme and general slowing of the blood stream; though, of course, such portal obstruction as may occur after adrenalin injection will be contributory, both through mechanical distention of vessels and through filtration of plasma, to the general slowing of blood flow due to constriction.

GENERAL SUMMARY AND CONCLUSIONS

1. The injection of adrenalin for a period of 20 to 30 minutes at such a rate as to maintain a high arterial pressure invariably constricts the arteries of both the somatic and the splanchnic areas. With large doses this constriction may be maximal and outlasts the injection period, it may be, for as long as 2 hours. This continued constriction is partly due to central action.

2. After sufficiently large doses, the arterial pressure, barring occasional intercurrent phenomena, falls steadily and slowly until the animal dies.

3. The jugular pressure, during or after large injections, shows no constant alteration, at least not of a kind that indicates inefficiency of the heart.

4. The heart may become irregular for a while and occasionally stops suddenly while the arterial pressure still is high.

5. The respiration after large doses often is slowed and may suddenly or gradually fail.

6. The portal pressure is increased, often markedly, during the injection, and may remain high subsequently; but not uncommonly it soon returns to the normal level.

7. The rise in portal pressure undoubtedly is caused by an obstruction in the liver which is so marked as to back up even the small amount of blood that is entering the portal system through the strongly constricted splanchnic arterioles.

8. The circulation may fail suddenly through stoppage of the heart due to direct action of the adrenalin, or to the indirect action of respiratory failure. But more often the failure of the circulation is gradual, the pressure steadily falling until the animal dies. The arterial constriction induced by the adrenalin then lasts until the end. In such instances a reduced blood volume, either real or effective, seems to be the main factor at fault. Apathy, as well as the other signs of shock, are present.

9. Evidence is presented indicating that accumulation of blood in the portal area as a result of the increased portal-hepatic resistance is not in itself the cause of the failure of the circulation. For marked obstruction of the hepatic radicles in the liver by the injection of a suspension of lycopodium spores may not lead to the shock-like failure of the circulation that is seen after adrenalin.

10. The failure of the circulation is rather to be attributed to the extreme slowing of the blood flow throughout the body caused by the constricting action of the adrenalin on the arteries. It is concluded that the cause of the failure is the same as is operative after temporary partial obstruction of the vena cava or of the aorta. This conclusion is justified by the fact that the most striking lesion found in animals dying as a result of any of these three procedures is alike, and consists of tremendous engorgement of the capillaries and venules of the villi of the intestines.

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PHYSIOLOGICAL STUDIES ON PLANARIA

I. Oxygen Consumption in Relation to Feeding and Starvation

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For a number of years the physiology of Planaria and other lower invertebrates has been extensively investigated in this laboratory. As a result of these investigations certain fundamental conceptions have been reached. These conceptions are in part concerned with the metabolic gradients of organisms and their relation to the phenomena of polarity, symmetry, individuation, regeneration, reproduction and development; and in part with the relation between metabolic rate and regeneration, age, starvation and other physiological conditions. These conclusions and the evidence from which they are derived have been presented by Professor Child in numerous papers and in his two books *Senescence and Rejuvenescence* and *Individuality in Organisms*.

In reaching these conceptions, various methods have been employed. Two of these are referred to in this paper and require explanation. One of them is called by us in the direct susceptibility method and consists in observing the time of death of organisms or parts of organisms in lethal concentrations of certain substances or under lethal conditions such as lack of oxygen, extreme temperatures, etc. A great variety of substances has been employed for this purpose, including cyanides, acids, bases, salts, anesthetics and dyes; all of them yield practically identical results, but we have found that cyanides constitute the most delicate and precise indicators of susceptibility differences. We believe that the time of death in such solutions or under such conditions is a direct measure of the metabolic rate of the protoplasm so tested. The chief grounds for this belief are: all conditions which are known to increase the general metabolic rate also increase the susceptibility to these solutions; in the case of the cyanides at least there is a large body of evidence, which I have reviewed in a recent

paper (1), which demonstrates that cyanides depress metabolic rate, and further we have recently shown (1), (2), that potassium cyanide decreases to a very marked degree both the carbon dioxide production and the oxygen consumption in Planaria; and finally the susceptibility data check completely with the results obtained by the second method referred to. This method consists in the determination of the carbon dioxide production of organisms or parts of organisms either by means of the Tashiro biometer or by means of an indicator, chiefly phenolsulphonephthalein. The carbon dioxide production furnishes the same conclusions regarding metabolic rates as does the susceptibility method, wherever comparable conditions are present.

In consideration of a misunderstanding which has arisen among other workers regarding the susceptibility method, it becomes necessary to emphasize the fact that the method in general measures only the susceptibility of the superficial structures of the body, since as a rule it is these only whose time of death can be accurately observed. Hence the time of death in these toxic solutions is, strictly speaking, a measure of the metabolic rate of the body wall and nervous system only and not of the total metabolic rate. It becomes a measure of the total metabolic rate only when comparable internal conditions exist. Hence when these internal conditions are altered as by introducing food into the intestine, the susceptibility of the surface will obviously not measure the metabolic rate of the intestine nor the total metabolic rate, since this is the sum of the activity of all parts of the body. Therefore the objections of Lund (3) to the susceptibility method on the grounds that in Paramecium the susceptibility is increased by short periods of starvation but the total oxygen consumption and carbon dioxide production are decreased are without basis and without point. In Protozoa obviously only the condition of the ectoplasm can be determined by the susceptibility method since as soon as the ectoplasm dies and disintegrates, the entoplasm scatters and can no longer be observed. In the cases of other organisms, however, it is sometimes possible to observe the time of death of both the intestine and the body wall separately and therefore to draw a correct conclusion regarding the total metabolism.

The expression "metabolic rate" seems to require definition. The word metabolism is generally taken to signify the sum of all the energy-producing and substance-producing processes occurring in the body. Each such process may, of course, have a rate of its own, and in order to find out the total metabolic rate at any one time it would be

necessary for strict accuracy to determine the rate of each process separately. This procedure is practically impossible and probably unnecessary, since it happens that in the animal body the majority of such processes are energy-consuming, that is, they use up oxygen and give off carbon-dioxide. It is therefore generally adequate to measure the rate of respiratory exchange in organisms in order to obtain some idea of the total metabolism. In the higher organisms, other methods are also at hand, as urine analysis, blood analysis, heat production, etc., which, however, cannot be applied to the lower forms. I wish to point out further that the rate of oxygen consumption and carbon-dioxide production are adequate measures of the rates of processes not in themselves essentially oxygen-consuming, such as the contraction of muscles; and that they furnish information about apparently unrelated conditions such as the irritability and conductivity of nerves. For these reasons we use the term metabolic rate instead of the more specific expression respiratory exchange, since we believe such respiratory rate to be an index of many other metabolic and physiological conditions besides oxidation. We also believe that the susceptibility method measures other metabolic conditions besides the rate of oxidation. We use the expression metabolic rate to indicate the rate of the large general chemical processes occurring in the body and not with reference to conditions specific to one organ or part nor with reference to special conditions. Thus the rate of the heart beat is an index to general physiological states such as age, sex, nutrition, etc., but obviously it would be foolish to attempt to draw conclusions regarding these states from the rate of the heart beat under special conditions where it had been altered, as after exercise. It is in precisely the same sense that we speak of metabolic rate, in reference to long-lasting and general processes, common to all of the cells of the body.

As previously stated, in our work on the physiology of the lower forms we have employed chiefly the method of differential susceptibility and the measurement of carbon-dioxide production. The present series of papers is concerned with the rate of oxygen consumption under various physiological conditions and therefore constitutes a check upon the results obtained by the other methods. The work has unfortunately been greatly hampered and delayed by the accidental loss in the spring of 1918 of our entire stock of Planaria, numbering many thousands. This has necessitated the use of somewhat smaller lots of worms than would ordinarily have been the case.

The present paper reports the results of my experiments on the rate of oxygen consumption of Planaria in feeding and starvation; experiments on oxygen consumption with respect to age and regeneration are also completed. It is gratifying to state that these results confirm the conclusions reached by the other methods.

METHOD

These experiments were performed upon *Planaria dorotocephala* and *Planaria velata*, two common species of flatworm, both of which have been used as the chief experimental material in the investigations mentioned in the opening paragraph. The method was the same as that described in a recent paper (1) except that, owing to the smaller stocks available, smaller receptacles and samples were employed. Briefly each lot of worms to be tested was placed in a 500 cc. Erlenmeyer flask filled air-tight with water, and allowed to respire in this flask at constant temperature for the desired length of time. A sample of the water was then withdrawn and analyzed for oxygen content by Winkler's method, and the difference between the oxygen content of this sample and a control sample similarly treated gave the oxygen consumption of the worms. In most cases where time and circumstances permitted, each test was repeated, so that two separate determinations of the oxygen consumption of a given lot of worms were obtained.

In order to compare the rates of oxygen consumption of the same lot of worms at different intervals it is necessary to know their weight. The oxygen consumption of these organisms is directly proportional to their weight, other factors being equal. This appears clearly in the experimental tables, and is in fact so axiomatic as to require no further discussion. The following method of weighing the worms was found most suitable. The animals were collected in a funnel of filter paper, being washed down to the point of the funnel by a pipette. After the water had drained through, the filter paper was opened out and spread flat on top of two or three layers of moist filter paper until all excess water had been absorbed, small pieces of dry filter paper being also placed under the mass of worms to aid the process. The worms were then scraped up with a dry scalpel blade into a small glass receptacle, previously balanced on the scales, and rapidly weighed. Weighings were made to the third place only. This method has been found more rapid and accurate than procedures in which the worms are

weighed in water, since it is almost impossible to avoid adding or withdrawing water. Usually some worms are injured through drying but as the weighings were nearly always made at the conclusion of the experiment, this was not of any consequence. If, however, it was necessary to weigh the worms before the experiment, the injured worms were collected and weighed separately and this weight then subtracted from the first weight. The injured worms were always discarded. Tests have shown that this method of weighing is sufficiently accurate for the purpose at hand. In these tests the same lot of worms was returned to water, re-collected and weighed again. Such duplicate weighings are not, however, strictly comparable, since the worms lose weight after weighing owing to the secretion of mucus due to the stimulation of drying. If this mucus is removed, the weight of the worms will be less than it was at the first trial; if retained, it may be more, as the mucus absorbs water. A fair idea of the loss due to the weight of the mucus can be obtained by drying it on filter paper and weighing it separately. The following are duplicate weighings of the same lot of worms: 0.192, 0.186 gram (mucus retained); 0.527, 0.532 (mucus retained); 0.458, 0.451 (mucus removed); 0.763, 0.746 (mucus removed, separate weight of the dried mucus, 0.014); 0.817, 0.802 (mucus removed, separate weight of the dried mucus, 0.014).

Eight lots of *Planaria dorotocephala* and three lots of *Planaria velata* were used in the experiments. Each lot, consisting of individuals of approximately the same size and condition, was selected from one of the general laboratory stocks. The oxygen consumption of each lot was then determined at various intervals after feeding and through a period of starvation, the lot being weighed after each such determination. The water was changed at intervals and the lots examined from time to time and all individuals noticeably larger or smaller than the average, all injured individuals and all posterior products of fission removed and discarded. When Planaria is placed in a clean receptacle, it is likely to undergo fission. This can be obviated to some extent by avoiding the use of clean dishes and placing the worms only in dishes previously occupied by worms. However, it is necessary to examine the dishes at intervals and pick out the products of fission. In the present experiments, all of the posterior products of fission have been removed and the anterior ones have been retained only when they constituted nearly the whole of the original worm. Thus these experiments deal only with whole worms of small size or anterior zoöids. As shown by Child's work (4), the posterior zoöids behave

somewhat differently during starvation than whole worms or anterior zoöids. As starving planarians will eat each other if possible, it is advisable to employ large receptacles for the starving stocks and to remove all individuals which vary much in size from the average of the stock. As starvation proceeds, the heads of the worms, especially if they were rather small individuals at the start, are likely to disintegrate, and new heads are then regenerated. Such individuals were removed during the early stages of the experiments but not at the end, as many of the individuals in some of the stocks were in this condition.

Since movement increases both the oxygen consumption and carbon dioxide production, movement is a disturbing factor in any experiments on the rate of respiratory exchange. In most of our experiments on Planaria, we remove the heads of the individuals to be tested several hours before the test, since decapitated worms remain perfectly quiet. This procedure was not thought advisable in the present experiments since the subsequent regeneration of the heads would introduce an additional factor. Hence it was necessary to observe carefully the degree of movement. If the worms are placed in the experimental flask several hours before the test, and if the flask is darkened during the test, movement can be largely eliminated. Movement is most likely to occur during the middle of the starvation period, recently fed worms or greatly starved worms being generally inclined to inactivity. Hence the figures obtained during the middle of the starvation period are probably slightly too high on account of movement. At the end of the starvation period at the last test made, in order to eliminate this factor completely, the heads were cut off, unless they had previously disintegrated as a consequence of starvation. Hence the final figures on the rate of oxygen consumption after prolonged starvation are entirely free from this difficulty.

Further details regarding the worms are given in the following records of the experiments.

EXPERIMENTAL RESULTS WITH PLANARIA DOROTOCEPHALA

Planaria dorotocephala is the species which has been employed in most of the investigations carried out by Professor Child and his students. Large stocks of this worm are kept constantly in the laboratory and fed three times a week on liver. From these general stocks eight lots, designated as A, B, F, G, H, K, M and N were selected

for the present experiments. The members of each lot were of approximately equal size and had been kept under the same conditions for some time preceding the experiments. Details regarding the make-up of each lot and the data obtained upon them are given in the following descriptions and accompanying tables. Six of the eight lots were fed and the effect of feeding as well as that of starvation on the oxygen consumption determined.

In the tables, the first column gives the length of time since feeding. The second column gives the actual figures of the oxygen consumption in a given period of time of the lot of worms on the day designated in the first column. In most cases two figures, the results of two separate determinations, are presented. The third column gives the weight of the lot of worms just after testing. The last column in each table is a calculation of the average oxygen consumed in cubic centimeters in two hours by 0.5 gram of Planaria. This calculation to a given weight and time is, of course, necessary in order to compare the rate of oxygen consumption at different periods of starvation. The time period and weight chosen for this calculation are, of course, purely arbitrary, selected as most nearly corresponding with the weights and time periods actually used in the experiments.

A word may be needed in explanation of the recorded weights. In the case of lots A, B, F, G and H, not all of the individuals in each lot were used each time in the early part of the experiments, a circumstance which accounts for the variability in weight. In lots K, M and N, the entire lot was used at each test, and the recorded weights therefore illustrate the loss of weight of these lots during starvation, with one increase due to feeding. I was somewhat at a loss to know how to regard the increase in weight resulting from ingestion of food. I finally decided that such increase should not be calculated in the results since this additional weight from the ingested food can hardly be regarded as respiring protoplasm, at least not within the first twenty-four hours after feeding. Hence in the feeding experiments the worms were weighed just before feeding, and this weight was used for calculating the rate of oxygen consumption after feeding.

The temperature of all experiments was 22 ± 0.5°C.

Record of lot A (*table 1*). Lot A consisted of worms about 10 mm. long selected from a stock which had been in the laboratory for four or five months (the exact date of collection was not known). They were last fed on March 3, 1919. On March 8, five days after feeding,

the average oxygen consumption of 0.5 gram in two hours was 0.30 cc.; on March 15, twelve days after feeding, it was 0.26; on March 29, after twenty-six days of starvation it was 0.36; and on April 29, after about two months' starvation, 1.50 cc. of oxygen was consumed. At this time when the experiment was concluded, the size and number of the worms was greatly reduced and most of them had lost their heads so that movement could not have played any rôle in the final result.

TABLE 1

Record of lot A

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED BY 0.5 GRAM IN TWO HOURS
days	*cc.*	*grams*	*cc.*
5	0.30 in 2 hours	0.464	0.30
	0.26 in 2 hours		
12	0.28 in 2 hours	0.499	0.26
	0.24 in 2 hours		
26	0.22 in 2 hours	0.302	0.36
57	0.15 in 3 hours		
	0.16 in 3 hours	0.034	1.50

TABLE 2

Record of lot B

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED BY 0.5 GRAM IN TWO HOURS
days	*cc.*	*grams*	*cc.*
5	0.46 in 2 hours	0.712	0.31
	0.42 in 2 hours		
12	0.26 in 2 hours	0.566	0.23
	0.28 in 2 hours		
26	0.23 in 2 hours	0.464	0.24
56	0.13 in 2 hours	0.147	0.47
	0.15 in 2 hours		

Record of lot B (table 2). Lot B was selected from a stock collected in February, 1919. They were slightly larger than the worms in the preceding stock, about 12 to 13 mm. long. The worms were last fed on March 5. On March 10, five days after feeding, the oxygen consumed in two hours by 0.5 gram was 0.31 cc.; on March 17, twelve days without food, it was 0.26 cc.; on March 31, after twenty-six days without food, it was 0.24; and at the end of the experiment, May

1, after nearly two months of starvation, it was 0.47. The worms were much reduced in size but had retained their heads. These were cut off several hours before the final test.

Record of lot F (*table 3*). This lot of worms was taken from a mixed stock which had been used for various other purposes. Worms in this stock had been collected during the fall and winter. The members of this lot were last fed on March 10. They were 12 to 15 mm. in length. On March 18, eight days after feeding, they consumed 0.25 cc. of oxygen in two hours as calculated for 0.5 gram weight. They

TABLE 3

Record of lot F

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED BY 0.5 GRAM IN TWO HOURS
	cc.	*grams*	*cc.*
8 days	0.17 in 1½ hour 0.17 in 1½ hour	0.444	**0.25**
Fed immediately after above determination			
1 to 3 hours	0.34 in 1½ hour	0.444*	**0.50**
3 to 5 hours	0.28 in 1½ hour	0.444*	**0.42**
2 days	0.18 in 1½ hour 0.19 in 1½ hour	0.486	**0.25**
7 days	0.21 in 2 hours 0.17 in 2 hours	0.413	**0.22**
21 days	0.12 in 2 hours	0.214	**0.28**
48 days	0.17 in 3 hours† 0.19 in 3 hours	0.075	**0.80**

* Weight taken before feeding, see text.

† Figures obtained on combined lots, F, G and H.

were then fed on liver and their oxygen consumption tested one to three hours after feeding. It was now 0.50 cc. Three to five hours after feeding it had fallen to 0.42 cc. It must be noted that the weight of these worms was taken before feeding and they were not weighed again after feeding as it was considered that the increased weight resulting from the intake of food could not properly be calculated with respiring protoplasm. It was therefore considered fairer to omit the increase in weight following feeding. On March 20, two days after feeding, the oxygen consumption had fallen to 0.25 cc.; on March 25, a week since feeding, it was 0.22; on April 8, three weeks of star-

vation, it was 0.28; and on May 5, when the experiment was concluded, after a fast of seven weeks, it was 0.80. The condition of the worms was similar to that in the preceding stocks. The number remaining was so few that it was necessary to combine those from lots G and H with those of lot F for the final determination on May 5. These three lots came from the same stock and had been treated in an identical manner throughout the course of the experiment.

Record of lot G (*table 4*). The worms in this lot came from the same stock as those of lot F, were of the same size and were handled

TABLE 4

Record of lot G

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
8 days	0.17 in 1½ hour 0.18 in 1½ hour	0.494	0.23
	Fed immediately after above determination		
1 to 3 hours	0.29 in 1½ hour	0.466*	0.40
3 to 5 hours	0.26 in 1½ hour	0.466*	0.37
2 days	0.13 in 1½ hour 0.20 in 1½ hour	0.423	0.26
7 days	0.17 in 2 hours 0.16 in 2 hours	0.344	0.24
21 days	0.11 in 2 hours	0.176	0.31
48 days	0.17 in 3 hours†	0.075	0.80
	0.19 in 3 hours		

* Weight taken before feeding, see text.

† Figures obtained on combined lots, F, G and H.

throughout in the same way. On March 18, eight days since the last feeding, they consumed 0.23 cc. of oxygen as calculated for 0.5 gram weight for two hours. They were then fed on liver, and one to three hours after feeding they consumed 0.40 cc.; three to five hours after feeding, 0.37 cc. On March 20, two days later, their oxygen consumption was 0.26; on March 25, one week after feeding, it was 0.24; on April 8, three weeks without food, it was 0.31; and on May 5, seven weeks without food, it was 0.80. As noted for lot F, this final figure was obtained on the combined individuals from lots F, G and H.

Record of lot H (*table 5*). This lot was the same as to origin, size and handling as the preceding lots F and G. On March 18, eight days since the last feeding, the oxygen consumption was 0.24 cc. in two hours per 0.5 gram weight. The worms were then fed, and during the first to third hour after feeding they consumed 0.44 cc. of oxygen; during the third to the fifth hour after feeding, 0.42 cc. Two days later, their oxygen consumption had fallen to 0.33 cc.; a week after feeding, it was 0.24; after three weeks without food, it was 0.31; and after seven weeks' starvation, it was 0.80. The three lots F, G and H

TABLE 5

Record of lot H

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
8 days	0.16 in 1½ hour 0.16 in 1½ hour	0.440	**0.24**
Fed immediately after above determination			
1 to 3 hours	0.28 in 1½ hour	0.421*	**0.44**
3 to 5 hours	0.27 in 1½ hour	0.421*	**0.42**
2 days	0.18 in 1½ hour 0.21 in 1½ hour	0.387	**0.33**
7 days	0.18 in 2 hours 0.16 in 2 hours	0.346	**0.24**
21 days	0.12 in 2 hours	0.192	**0.31**
48 days	0.17 in 3 hours† 0.19 in 3 hours	0.075	**0.80**

* Weight taken before feeding, see text.

† Figures obtained on combined lots, F, G and H.

were, as can be readily seen, run simultaneously and were always under the same conditions. At the end of the starvation experiment they were greatly reduced in size and most of them had lost their heads, thus eliminating movement as a factor in the final determination.

Record of lot K (*table 6*). Lots K, M and N were three lots selected from the same stock and run in all respects under the same conditions. These three lots were tested with especial care, and the data presented for them are hence possibly more exact than the data for the five preceding lots. They were selected from a new stock, collected on March 11, 1919. They were last fed on March 24. The worms of

these lots were larger than in the preceding cases, 15 to 18 mm. long. In consequence of this larger size, they were not reduced so much at the end of the experiment, and all had retained their heads throughout the period of starvation. The possible disturbing factor of regeneration of the head is therefore eliminated in the case of these three lots. These worms were also so active at the conclusion of the experiment that it was necessary to decapitate them before testing their rate of oxygen consumption. In these three lots then movement has been completely eliminated as a factor in the final results. In short, the

TABLE 6

Record of lot K

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
4 days	0.23 in 1½ hour	0.589	**0.25**
	Fed immediately after above determination		
2 to 4 hours	0.46 in 1½ hour	0.579*	**0.52**
7 to 8½ hours	0.48 in 1½ hour	0.579*	**0.54**
24 hours	0.30 in 1½ hour	0.579*	**0.32**
3 days	0.32 in 2 hours	0.623	**0.25**
7 days	0.27 in 2 hours	0.543	**0.24**
21 days	0.21 in 1½ hour†	0.325	**0.38**
	0.16 in 1½ hour		
48 days	0.11 in 2 hours	0.112	**0.53**
	0.13 in 2 hours		

* Weight taken before feeding, see text.

† Considerable movement during this determination.

data for these three lots seem to me to be absolutely dependable and to be free from all possible sources of error. The entire stock was used in each case for each determination, and hence the weights given are a fair picture of the loss of weight of Planaria during starvation. Of course a few individuals were discarded from time to time on account of injury. Fission, however, was practically absent in these lots.

The oxygen consumption of lot K on March 28, four days after feeding was 0.25, calculated as before for two hour periods and for 0.5 gram of weight. The worms were then fed and the oxygen consumption two to three and a half hours after feeding was 0.52; seven

to eight and a half hours after feeding it was 0.54. On the next day, twenty-three to twenty-four hours after feeding, it was 0.32. On March 31, about three days after feeding, it was 0.25; on April 4, a week without food, it was 0.24; April 18, three weeks without food, it was 0.38; and at the conclusion of the experiment, on May 15, after seven weeks' starvation, it was 0.53. It should be noted that the worms were rather active during the determination of April 18, and hence the figures obtained upon this date are slightly too great, particularly the first of the two successive determinations. During the

TABLE 7

Record of lot M

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
4 days	0.29 in 1½ hour	0.761	**0.25**
	Fed immediately after above determination		
2 to 4 hours	0.58 in 1½ hour	0.747*	**0.51**
7 to 8½ hours	0.54 in 1½ hour	0.747*	**0.48**
24 hours	0.37 in 1½ hour	0.747*	**0.33**
3 days	0.43 in 2 hours	0.779	**0.28**
7 days	0.32 in 2 hours	0.671	**0.23**
21 days	0.23 in 1½ hour†	0.424	**0.31**
	0.17 in 1½ hour		
48 days	0.13 in 2 hours	0.159	**0.45**
	0.16 in 2 hours		

* Weight taken before feeding, see text.

† Considerable movement during this determination.

second analysis they were for the most part inactive and hence this figure is more nearly correct. As already stated the heads of the worms were cut off for the final determination so that movement was completely eliminated.

Record of lot M (*table 7*). All the remarks made with reference to lot K apply to this lot also. Four days after the last feeding, lot M consumed 0.25 cc. of oxygen; on the same day, two to three and one-half hours after feeding, this figure rose to 0.51 cc.; and seven to eight and one-half hours after feeding, it had fallen slightly to 0.48. The next day it was 0.33 cc.; on the third day after feeding, 0.28; after a

week without food, 0.23; after three weeks, 0.31; and after seven weeks' starvation, 0.45.

Record of lot N (*table 8*). This lot is similar in all respects to the two preceding lots. These worms consumed on the fourth day after feeding 0.23 cc. of oxygen per 0.5 gram weight in two hours; on the same day shortly after feeding, the oxygen consumption rose to 0.51, and fell a few hours later to 0.49. The next day it was 0.31; the third day after feeding, 0.27; a week after feeding, 0.22; after three weeks without food, 0.28; and after seven weeks without food, 0.56.

TABLE 8

Record of lot N

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
4 days	0.27 in 1½ hour	0.769	**0.23**
Fed immediately after above determination			
2 to 4 hours	0.58 in 1½ hour	0.758*	**0.51**
7 to 8½ hours	0.56 in 1½ hour	0.758*	**0.49**
24 hours	0.36 in 1½ hour	0.758*	**0.31**
3 days	0.45 in 2 hours	0.832	**0.27**
7 days	0.32 in 2 hours	0.706	**0.22**
21 days	0.19 in 1½ hour 0.19 in 1½ hour	0.446	**0.28**
48 days	0.14 in 1½ hour 0.14 in 1½ hour	0.125	**0.56**

* Weight taken before feeding, see text.

EXPERIMENTS WITH PLANARIA VELATA

Planaria velata is a turbellarian flatworm found in temporary pools in the spring in the Chicago region. Associated with the peculiar conditions of life which exist in temporary pools, the life cycle of this animal is exceptionally interesting. It has been investigated by Child, to whose paper on the subject the reader is referred (5). The animal emerges from cysts in the spring when the temporary ponds fill with water, grows rapidly and soon attains adult size. It then ceases to feed and posterior portions of its body drop off, secrete a mucous cyst about themselves, and pass into a quiescent state within this cyst.

This process continues until the entire worm has formed a series of cysts. After about four weeks, in case the pond still contains water, the worms emerge from the cysts, but they are now completely regenerated whole worms of small size. This cycle is repeated until the pond dries up, whereupon the cysts remain quiescent until the following spring.

I performed three experiments with this species. In each case the experiment was interrupted by the formation of cysts. The young worms which emerge from the cysts are of course in a state of extreme starvation. As, however, not all of them emerge at once, it was necessary to feed those which emerged first in order to keep them alive until a number of individuals sufficient for a determination of their oxygen consumption had appeared. The young starved worms were therefore fed two or three times at long intervals. Since this feeding was entirely inadequate to bring about growth, it is certain that at the time of testing the young worms were still in a state of extreme starvation, and that their oxygen consumption therefore truly represents starvation metabolism. In addition it must be noted that these worms have also undergone regeneration. Now as I shall show in the second paper of this series, regeneration also greatly increases the rate of oxygen consumption. Hence, in these experiments with *Planaria velata*, the end result is a combination of the effects of both starvation and regeneration on the metabolic rate. It is not in this particular case possible to separate these two factors, although one can prevent this species from undergoing encystment by beginning to starve the worms before they have attained adult size. This I attempted to do but evidently growth had already progressed too far when I began the experiments and all of the worms encysted.

Record of lot C (table 9). This lot of *Planaria velata* was taken from a collection of March 7, 1919. They were fed once after being received in the laboratory, on March 10. The individuals selected for the experiment were 10 to 12 mm. long. On March 13, three days after feeding, they consumed 0.31 cc. of oxygen in two hours per 0.5 gram weight. On the same day, two to three and one-half hours after feeding, the oxygen consumption was 0.43. It should be noted that only a small proportion of the lot fed, as they were evidently already on the verge of encystment. On March 15, two days after feeding, the oxygen consumption was 0.32; on March 20, a week without food, it was 0.29. The worms now rapidly encysted and in a week or two the dishes were a mass of small round cysts. Late in

TABLE 9

Record of lot C

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
3 days	0.32 in 1½ hour 0.42 in 1½ hour	0.745	**0.31**
Fed immediately after above determination			
2 to 3½ hours	0.46 in 1½ hour	0.707*	**0.43**
2 days	0.28 in 1½ hour 0.30 in 1½ hour	0.602	**0.32**
7 days	0.19 in 1½ hour	0.428	**0.29**
Worms encysted and emerged as young worms, see text			
75 days	0.10 in 3 hours 0.14 in 3 hours	0.058	**0.68**

TABLE 10

Record of lot D

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
3 days	0.44 in 1½ hour 0.39 in 1½ hour	0.774	0.35
Fed immediately after above determination			
2 to 3½ hours	0.50 in 1½ hour	0.754*	0.49
2 days	0.37 in 1½ hour 0.37 in 1½ hour	0.648	0.38
7 days	0.22 in 1½ hour	0.525	0.27
Worms encysted and emerged as young worms, see text			
70 days	0.19 in 3 hours 0.21 in 3 hours	0.140	0.70

*Weight taken before feeding, see text.

April and throughout May, the young worms were emerging. They were picked out on May 3, and fed at that time; then again on May 10, and fed; and again on May 21. On May 26, those which had emerged since were added to the lot of young worms and at this time the heads of all were cut off to prevent movement. On May 27, the final test of the rate of oxygen consumption was made. It should be noted therefore that lot C at that time was made up of young worms, some of which had been fed three times, some twice, some once, and some not at all. As already noted the feedings were at such long intervals that the worms were in a state of starvation. The oxygen consumption on May 27 was 0.68.

Record of lot D (*table 10*). This lot of worms was identical in make-up and kept under the same conditions as the preceding lot. On March 13, three days after feeding, the oxygen consumption was 0.35 cc.; on the same day a few hours after feeding (most of the worms refused to feed), it was 0.49. Two days later, it was 0.38; and after a week without food, it was 0.27. Encystment now ensued as related in the preceding case. The emerged worms were fed on May 3 and May 10 and tested on May 22, so that the lot finally tested contained some worms which had been fed once, some twice and some which had not received any food. The oxygen consumption on May 22 was 0.70 cc. The heads were not removed but the worms certainly were no more active than in the earlier tests on this lot, so that the results are dependable.

Record of lot E (*table 11*). This lot is identical with lots C and D. On March 13, three days after feeding, the oxygen consumption was 0.34; on the same day, a few hours after feeding, it rose to 0.53. More of the individuals in this case took food than in the case of the two preceding lots. Two days later, the oxygen consumption had fallen to 0.35 cc.; and a week after feeding it was down to 0.21. After emergence from the cysts, the young worms were fed on May 3 and May 10, and tested on May 22, some of the lot at the time of the test having therefore fed twice, others once, and others not at all. On this day, the oxygen consumption was 1.05.

On May 26, all worms which had emerged in lots C, D and E since May 22 were collected into one lot and their heads removed. No members of this lot had been fed, and they had therefore been without food for seventy-five days. Their rate of oxygen consumption was tested on May 27. They consumed 0.16 and 0.17 cc. of oxygen in two tests, each lasting three hours. They weighed 0.067. The

oxygen consumption as calculated for two hours and for 0.5 gram weight was 0.80. In this experiment, then, movement had been completely eliminated by decapitation, and none of the worms had received food. The rate of oxygen consumption of such emerged starved worms was more than twice as high as that of the same worms a few days after feeding. Both regeneration and starvation are involved in this increase, as already noted.

TABLE 11

Record of lot E

TIME SINCE FEEDING	OXYGEN CONSUMED IN TEST	WEIGHT	CALCULATION, OXYGEN CONSUMED IN TWO HOURS BY 0.5 GRAM
	cc.	*grams*	*cc.*
3 days	0.31 in 1½ hour	0.593	0.34
Fed immediately after above determination			
2 to 3½ hours	0.47 in 1½ hour	0.581*	0.53
2 days	0.29 in 1½ hour 0.24 in 1½ hour	0.494	0.35
7 days	0.12 in 1½ hour	0.374	0.21
Worms encysted and emerged as young worms, see text			
70 days	0.18 in 3 hours 0.20 in 3 hours	0.60	1.05

* Weight taken before feeding, see text.

CONCLUSIONS AND DISCUSSION

The data obtained from eleven different lots of Planaria, belonging to two species, are in complete agreement with each other and serve to demonstrate the following facts regarding the oxygen consumption of Planaria during feeding and starvation.

1. The oxygen consumption is increased after feeding. The amount of this increase depends directly upon the number of individuals which take in food. Thus in the case of lots K, M and N, presented in tables 6, 7 and 8, where practically all of the individuals fed, the increase is more than 100 per cent, while in lots F, G and H, in which some individuals did not feed, the increase ranges from 80 to 100 per cent, and in lots C and D, where the majority of the individuals

refused to feed, the increase is only 40 per cent. In lot E, worms comparable in every way to those of lots C and D, more of the worms were observed to apply themselves to the meat and a correspondingly greater increase, about 60 per cent, in rate of oxygen consumption was found.

2. This increase in the rate of oxygen consumption due to feeding begins to fall within a relatively short period of time after feeding. A slight decrease is noticeable seven to eight hours after feeding, and twenty-four hours after feeding the rate has fallen off very markedly. The rate continues to fall more slowly from this time on and reaches a minimum value one to two weeks after the last feeding.

3. After the rate of oxygen consumption has reached a certain minimum value, it begins to rise. This rise is distinctly noticeable three weeks after feeding.

4. The oxygen consumption continued to rise as long as the experiments were continued. After seven to eight weeks without food, the oxygen consumption is two to five times as great as it is in the same worms several days after the last feeding.

5. Starvation is therefore a means of increasing the rate of oxygen consumption of Planaria. After a long period of starvation the organism has a metabolic rate like that of young worms and resembles them in shape and proportions, color, rapidity of movement and in general reactions and behavior. Child has shown (4) that the carbon-dioxide production of young worms is invariably greater than that of old worms per unit weight. I have recently found (results to be published shortly) that similarly the oxygen consumption of young worms is invariably higher than that of old worms, per unit weight, both being in a condition of adequate nutrition. The difference between fed young and old worms is not so great as that between fed and starved worms. Starved worms are therefore metabolically like young worms, when both are compared with old fed worms.

These conclusions merit further discussion, with reference to experiments on Planaria by other methods and experiments on other forms.

The effect of feeding and starvation on *Planaria dorotocephala* and *Planaria velata* has already been studied by Child by means of the susceptibility method and through carbon-dioxide production (4), (5), (6), (7). A significant difference exists between the results obtained by these two methods. The susceptibility continually increases during the period of starvation and is not increased by feeding, while both the carbon-dioxide production and the oxygen consumption

are increased by feeding, decrease during the earlier stages of starvation, and increase only in the later stages of starvation. As already emphasized in the introductory remarks, susceptibility concerns chiefly the metabolic condition of the superficial structures of the body. The metabolic rate of these structures is then not increased by feeding but increases throughout the period of starvation. On the other hand, the susceptibility of the intestine, which can be observed separately in Planaria, is increased by feeding, falls after feeding, and rises again in the later stages of starvation. The susceptibility of the intestine therefore runs parallel to the total metabolism as measured by both carbon dioxide production and oxygen consumption. It is therefore certain that the increase in the rate of respiratory exchange seen after feeding in Planaria is due entirely to the increased activity of the digestive apparatus and the subsequent decrease in total metabolism during the early stages of starvation is due to the decreased activity of the digestive tract.

The data at hand permit us to draw certain conclusions as to the factors involved in the increase of metabolic rate after feeding. I have already mentioned that the effect of feeding on total metabolism decreases rapidly so that within twenty-four hours after a single feeding, the rate had fallen to a marked extent. Now it is certainly impossible for Planaria to digest a meal completely in this short length of time. The intestine of Planaria remains filled with food undergoing digestion certainly for as long as a day after feeding and probably much longer. If Planaria is fed on blood clot, the corpuscles can still be recognized within the intestine on the following day. If these worms are fed at intervals of two days, only a portion of them will take food on each occasion. Hence it is fairly certain that in Planaria the rise in rate of oxygen consumption after feeding cannot be attributed to assimilation or similar anabolic processes, since such processes would be going on even more rapidly twenty-four hours after feeding than a few hours after feeding; yet by the next day the oxygen consumption has fallen markedly. Further any increase in assimilative processes ought to be detectable in other parts of the body also but the susceptibility method demonstrates that the metabolic rise following feeding involves the intestine only. One may therefore conclude that in Planaria the increase in metabolic rate following feeding is attributable to the activity of the intestine, including probably such processes as the secretion of digestive enzymes by the entoderm, amoeboid movements of the entoderm cells, and absorption of food through the entoderm, all processes which

are known to involve an increased rate of respiratory exchange. It is probable, however, that all of these activities combined are not adequate to account for the increase observed. One must therefore postulate a *direct stimulation* of the entoderm cells by the food or its products, causing an increase in the intrinsic metabolic rate of these cells, not due either to formation of new protoplasm or to oxidation of the food products, although the latter process may of course play some rôle. A similar conclusion has been reached in regard to mammals, as will be considered below.

All three methods, susceptibility, carbon-dioxide production and oxygen consumption, demonstrate that in Planaria the total metabolism is markedly increased in long-continued starvation. The relative susceptibilities of fed and starved animals have been tested by Child not only with cyanide, alcohol and other anesthetics, but also recently with lack of oxygen, a method to which none of the objections possible with the toxic solutions can be raised. It may therefore be concluded that starved worms are in a metabolic condition identical with that of young worms, and that starvation is a means of rejuvenescence. This matter has been so fully treated in Child's book that further discussion of it is superfluous.

The oxygen consumption in starvation rises much more rapidly than does the carbon-dioxide production, and runs more in accord with the results obtained by the susceptibility method than does the latter. It may therefore be suggested that the chemistry of the oxidation processes is different during starvation than in a condition of adequate nutrition. The oxygen consumption rises but the carbon-dioxide production increases less rapidly so that the organism is apparently carrying on oxidations which do not involve evolution of carbon-dioxide. Perhaps this is due to the fact that it is using its own tissues as a source of energy while ordinarily it is using ingested food. It seems reasonable to suppose that the former can be burned more economically than the latter.

Experiments on the metabolism of other animals in relation to feeding and starvation are scanty in number, with the exception of mammals. Among the Protozoa, tests have been made on Paramecium only. Barratt (8) first showed that the carbon-dioxide production of Paramecium is decreased during the early stages of starvation. Recently Lund (3), (9) has confirmed this observation and has performed more extended experiments on the rate of carbon-dioxide production and oxygen consumption in feeding and early starvation in this organism.

The rates of both processes were increased by feeding and decreased in early starvation. Susceptibility to cyanide (of the ectoplasm) was not increased by feeding but increased in starvation. The rise in metabolic rate following feeding is therefore as in Planaria due solely to the activity of the entoplasm, and the fall in rate in early starvation is attributable to decreased activity of the entoplasm. The results with Paramecium as far as they go are therefore identical with our results on Planaria. Lund's experiments were not carried on for a sufficient length of time to determine whether or not there is an increased metabolism in prolonged starvation; this is probably impractical as one would have to know the weight of the organisms. Lund states that the increase in metabolic rate after feeding is the result of "ingestion, digestion and assimilation of the food by the cell leading to growth." It is probable that the activity involved in Paramecium in sweeping food into the gullet and in the formation of food vacuoles would account for a small part of this increase. Digestion may be ruled out, as digestion is chemically a process of hydrolytic cleavage involving neither oxygen consumption nor carbon-dioxide production. It is probable that what Lund really means is the secretion of digestive enzymes, an activity undoubtedly involving an increase in respiratory exchange. Whether assimilation is concerned in the result cannot be decided from the data presented, since the animals were supplied with food throughout the experiment and were continually ingesting food throughout the experiment. If assimilation and growth are really involved in the increased metabolism noted, then they must affect the ectoplasm also. However, the susceptibility method showed that the metabolic rate of the ectoplasm was not increased by feeding, but only by starvation. It may further be pointed out that assimilation as commonly understood and certainly that resulting in the formation of new protoplasm is chemically a process of dehydration, involving no increase in rate of respiratory exchange. It seems very likely therefore that in Paramecium the factors mentioned by Lund are not adequate to account for the increased metabolism observed in feeding but that as in Planaria and as has been concluded in regard to mammals, a direct stimulation of the digestive apparatus by the food is involved. Oxidation of the products of digestion may be a factor also.

The effects of feeding and starvation on Hydra have been tested in this laboratory but by the susceptibility method only. We have found (10) that in this animal the ingestion of food causes a local

increase in susceptibility. This increase is confined solely to the small region of the column which contains the food and the susceptibility of the rest of the animal is unaffected. Soon after ingestion, the susceptibility falls in this local region. The susceptibility of the entire animal is greatly increased by starvation (one or two weeks), and such starved animals resemble in susceptibility and behavior newly released buds. Conditions in Hydra are therefore like those in Planaria.

Some data on other coelenterates are available also through the work of Vernon (11). In some of these forms the rate of respiratory exchange was tested on successive days without food but the experiments were unfortunately carried on for a few days only. In two species of ctenophores and two species of Salpa (a pelagic tunicate), the rate of respiratory exchange was found to increase daily when the organisms were kept without food. Such an immediate increase in the absence of food has not been noted in other organisms but may be accounted for in these forms by their extreme delicacy and lack of solid structures which do not permit the storage of excess food; they are hence probably in a state of starvation very soon after cessation of feeding. In other similar coelenterates, two kinds of medusae, the rate of respiratory exchange remained about the same during the period tested, three to four days without food. In these watery coelenterates, then, the metabolic rate ceases to fall or may even rise in very short periods of starvation.

In other marine organisms, nudibranchs, octopus, Amphioxus and teleosts, Vernon noted a fall in metabolic rate in the early days of starvation. These forms, whose anatomy permits of food storage, are therefore similar to Planaria, as far as the data permit a comparison.

For some time I have been studying the effect of starvation on the metabolic rate of the smaller aquatic oligochaetes, chiefly Tubifex. In this worm, the susceptibility to cyanide increases continuously throughout a period of starvation extending over two months. The carbon-dioxide production, however, falls in the early days of starvation, and increases later. This increase occurs much earlier than in the case of Planaria, a fact which we attribute to the simpler structure of the digestive tract in Tubifex as compared with Planaria. In general, however, except for this detail, the metabolic conditions in these worms, in relation to starvation, are like those found in Planaria.

In fish, Wells (12) has tested the susceptibility to cyanide during starvation. The susceptibility was found to decrease during the early

part of the starvation period and to increase subsequently. Here, contrary to the findings on the lower organisms, the decrease in metabolism in early starvation shows up with the susceptibility method. In the lower forms, it is the susceptibility of the intestine alone which exhibits such a decrease. In these lower forms, susceptibility is measured by the time of death and disintegration of the body surface; in fish by the time of death of the organism as a whole. In this latter case, as Child has suggested, the time of death is probably a more definite measure of the total metabolic rate, since in vertebrates, the cyanide circulating in the blood causes death chiefly by its effect upon internal parts. In fish, then, as in lower invertebrates, the metabolic rate is increased in prolonged starvation.

The data upon mammals are so extensive that it would be out of place to attempt to review them in this brief paper. They are adequately presented in Lusk's *The Science of Nutrition*, in his various papers on animal calorimetry published in the *Journal of Biological Chemistry*, and in the publications of the nutrition laboratories at Middletown and Boston. This work on mammals agrees with the work on the lower forms. In mammals, the metabolic rate is increased after the ingestion of food. This increase is greatest in the case of ingestion of protein, less in the case of carbohydrates or fats. The cause of the increased metabolism after protein ingestion has been carefully investigated by Lusk and his associates (13) who came to the conclusion that a direct stimulation of the cells by the metabolic products of amino-acids was responsible for the results. Only certain of the amino-acids have this "specific dynamic action." Neither processes of deamination nor direct oxidation of ingested protein by the cells could be regarded as responsible since the increase was greatest shortly after ingestion of the protein or amino-acids before a sufficient time had elapsed, as shown by urinea nalysis, for the metabolizing of the ingested materials to have occurred. Some carbohydrates, such as glucose, also increased the metabolic rate, and the same was true of fat. In both of these cases, the increase was probably due to an actual oxidation of the foods after they had reached the cells (14). In mammals, then, ingestion of food almost invariably increases the metabolic rate, either through a direct stimulating effect of the constituents of the food upon the cells, or through the oxidation of the newly available nutritive molecules. The activity of the digestive glands and digestive apparatus apparently plays a negligible rôle in the observed metabolic increase.

In mammals as in other forms the metabolic rate soon begins to fall after ingestion. In the experiments quoted above the maximum effect in the case of protein food was observable two to three hours after ingestion, remained high up to about fourteen hours after ingestion, and then gradually fell. The carbon-dioxide production and oxygen consumption began to fall within eight to ten hours after ingestion. In the case of carbohydrate food, the increase is at the maximum during the second to fifth hours after ingestion, and in the case of fat attains a maximum by the sixth hour.

Studies on prolonged fasts in mammals have shown that the metabbolism continues to fall during the early days of the fast. Soon however the metabolism attains a nearly constant level and after that begins to rise. The carbon-dioxide production, the oxygen consumption and the total heat production, as calculated per kilogram of body weight, rise in the later periods of prolonged fasts. These data are given in Benedict's report on a fast of thirty-one days by the subject L (15) and in this publication similar results are reported from prolonged fasts in dogs. In L, it is also important to note that the rate of the heart beat was distinctly accelerated toward the end of the fast.

In mammals, therefore, including man himself, the effect of starvation is the same as in Planaria and other lower organisms. As a result of starvation, all organisms are restored to a metabolic condition resembling that of younger animals. It has been the general impression of men who have undergone long fasts, and observations on dogs confirm this, that they are in a better physiological state after than before fasting.

One point still remains to be discussed. It might be maintained that the increased metabolism per unit of weight observed in fasting is only apparent, since it might be supposed that the decrease in weight was due solely to a loss of non-respiring materials. If, however, this were the case, the metabolism as calculated per unit body weight ought to increase in the early days of the fast when this non-respiring material is disappearing most rapidly. This, however, is not the case. Further, the heart loses less material than almost any other part of the body, and hence the increase in the rate of the heart beat in starvation cannot be attributed to loss of non-respiring materials but must represent a real increase in the basic metabolism of the heart protoplasm. That the intensity of cellular activity is really increased in fasting is the conclusion reached by Benedict from his study of the metabolism of L in fasting.

The increased cellular metabolism in fasting is probably, as Child has long maintained, due to the removal from the cells of reserves, structures and deposits of one kind or another which interfere with metabolic processes. The removal of these hindrances to metabolism restores the cell to an undifferentiated condition like that of young cells and permits a metabolic activity like that of such cells.

SUMMARY

1. The oxygen consumption of Planaria is increased markedly after the ingestion of food.

2. This increase is maintained only for several hours after ingestion of food, and the oxygen consumption then begins to fall. By the following day the metabolism has decreased again to a marked degree.

3. The oxygen consumption continues to fall in the early days of starvation, reaching a minimum value within the first two weeks.

4. The oxygen consumption then begins to rise and at the end of a period of prolonged starvation it is much higher than in animals starved only a few days.

5. Data quoted from the work of other investigators show that in other organisms the effects of feeding and starvation on metabolic rate are essentially the same as in Planaria.

6. It may therefore be concluded that starvation increases the metabolic rate of organisms, and that starved organisms are metabolically in a condition similar to that of young organisms.

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SUSCEPTIBILITY TO LACK OF OXYGEN DURING STARVATION IN PLANARIA

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In the course of investigation of the physiological condition of *Planaria dorotocephala* during starvation, the following facts have already been ascertained and recorded in earlier papers: first, that the susceptibility of ectoderm and body wall to concentrations of cyanide and various other agents which are lethal within a few hours increases during starvation (1), (3); second, that susceptibility of the alimentary tract to the same agents decreases rapidly in relation to that of the body wall during the earlier stages of starvation, but later shows some increase (5); third, that CO_2 production also decreases rapidly at first, but later shows some increase (5); fourth, that starved animals appear to be extremely sensitive and show distinctly less capacity for acclimation to certain low concentrations of cyanides and other agents than fed animals, this capacity decreasing during the course of starvation (1), (3). Numerous facts indicate that susceptibility to cyanides and many other toxic agents in sufficient concentration to kill without permitting any marked degree of acclimation or development of tolerance is in a general way and under at least a wide range of conditions, a rough criterion of metabolic condition, the susceptibility varying directly with, though not necessarily proportionally to the rate of metabolism or probably oxidation (2), (3), (4). Consequently the increase in susceptibility of ectoderm and body wall during starvation suggests that the rate of oxidation in these organs increases as starvation progresses, while the decrease in susceptibility of the alimentary tract during the early stages of starvation suggests a decrease in rate in this organ in the absence of food. It has been pointed out in another paper (5) that the data on susceptibility and those on CO_2 production during starvation are not in conflict when the alimentary tract is taken into account. It has been shown that the marked decrease in CO_2 production during the first few days of starvation is evidently due, at least in large part, to decrease in the

metabolic activity of the alimentary tract in the absence of food, and that in advanced stages of starvation, when the alimentary tract, as well as other organs, is undergoing reduction, there is a distinct increase in total CO_2 production. The conclusion suggested by the experimental data on susceptibility and CO_2 production is that ectoderm and body wall, which maintain or even increase their functional activity during starvation, show an increase in oxidation rate, while the alimentary tract at first undergoes a marked decrease in rate in consequence of decreased function, but later, when undergoing reduction, may also show some increase in rate. According to this interpretation, the increase in susceptibility of ectoderm and body wall during starvation and the decrease in CO_2 production during earlier stages, followed by increase in later stages, are not in any way contradictory. Susceptibility of ectoderm and body wall is a criterion of conditions in these parts only, while CO_2 production is a measure of conditions in the body as a whole. Since the CO_2 production of the alimentary tract is evidently a large part of the total, the changes in the alimentary tract are the chief factor in determining the changes in total CO_2, and only in later stages of starvation, when the alimentary tract is undergoing reduction, is the increase in CO_2 production sufficient to balance the earlier decrease, resulting from decrease in functional activity in the alimentary tract. At present this seems to be the only logical and satisfactory interpretation of the facts.

Whatever the course of the changes in physiological condition during starvation in *Planaria*, it is certain that animals which are again fed after a period of starvation are, as regards susceptibility, capacity for acclimation, CO_2 production, rate of growth and all other distinguishable characteristics, physiologically younger than at the beginning of the starvation period (1), (3) (4, chap. VII), (5), being in approximately the same physiological condition as well-fed growing animals of their own size or somewhat smaller. In other words, the decrease in size during starvation serves roughly as a measure of the degree of regression or rejuvenescence which the animals have undergone.

The present paper is concerned with another aspect of the problem of starvation and its effects in *Planaria*, viz., the changes in susceptibility to lack of oxygen. Since oxygen is essential to the life of *Planaria*, susceptibility to lack of oxygen may be expected to show some relation to the fundamental metabolic condition. Moreover, various authors have observed that the action of cyanides seems in certain respects to be similar to that of lack of oxygen and I have found that cyanide in-

creases susceptibility to lack of oxygen, i.e., cyanide and lack of oxygen are additive in their action (7). And finally, susceptibility to lack of oxygen is the reaction of the animal to the absence of a factor essential for its fundamental metabolism, rather than to the presence of a toxic agent, and the possible complicating conditions involved in the use of toxic agents for determination of susceptibility are absent.

METHOD

Since the chlorination of the Chicago city water supply renders this water injurious to *Planaria*, water pumped from a well is used for all stocks and all experiments with the animals. This water, which before exposure to the air has a very low oxygen content, usually $\frac{1}{10}$ cc. per liter or less, has served as the basis for the experiments on susceptibility to lack of oxygen, but the procedure has been modified in various cases by introducing more or less oxygen into the water and thus prolonging the survival time.

The usual method of procedure is as follows: the animals to be compared, e.g., well-fed animals and animals starved a certain length of time, or starved animals and animals fed after starvation, etc., are placed together in a pyrex tube about 10 mm. in diameter, closed by fusion at one end, holding 5 to 6 cc. of water and calibrated in cubic centimeters, and this tube is sealed without air bubbles at any desired level, so that the animals are confined in 1, 2 or more cubic centimeters of water. Since the data are purely comparative, the placing of the two or more lots to be compared in the same tube provides identical conditions as regards oxygen for all the animals and avoids many complications. Since the animals to be compared in each case are in the same water it maks no essential difference whether the different lots are of equal weight, and in consequence of the oxygen consumption of all the animals in the tube the oxygen concentration is undergoing continuous decrease and the most susceptible animals must die first. Of course the actual survival time of the lots and the amount of difference between them will vary with different conditions in the tubes but the essential point, i.e., whether one lot is more or less susceptible than another, is readily determined by this method. It is not even necessary to know the oxygen content of the water in a particular experiment, if the precautions mentioned below concerning CO_2 are observed.

Several methods of sealing the tubes have been used. One of these which avoids exposure of the water to air, consists in the insertion of

a small tight plug of absorbent cotton to any desired level in the tube while both tube and cotton are held well below the surface of a large volume of the water coming directly from the pump and then sealing above the cotton with melted paraffine. Since leakage occasionally occurs around the paraffine, particularly if changes in temperature occur, the entrance of air is prevented by placing a tightly fitting soft rubber tube 2 to 3 cm. long over the open end of the glass tube, filling both glass and rubber tube with the water used and closing with a screw clamp. Air may of course be excluded by the rubber tube and clamp alone, but the animals are likely to creep into the rubber tube out of the light and die there, and since they tend to stick to surfaces when they begin to die they cannot readily be removed to the glass tube, where they can be seen. The cotton plug serves merely to avoid exposure of the water in the tube to the air and to remove the possibility of injuring the animals by temperature changes when the melted paraffine is run in. If care is taken to keep the animals at the bottom of the tube the paraffine may be run in directly on the surface of the water, though this necessitates exposure of this small surface to the air for a short time.

In most cases lots of 3 to 5 worms each are used, and when starved and well-fed animals are compared there is no difficulty in distinguishing the animals of the two lots, even when they are of the same size. In cases where it may be difficult to distinguish animals of different lots, the tips of the posterior ends of one lot may be cut off. This or some other slight injury does not alter the susceptibility to any appreciable degree, except in the immediate region of the injury.

Since the animals not only consume oxygen, but produce CO_2, it is necessary to make certain that death results from lack of oxygen and not from accumulation of CO_2. The well water when first pumped contains a slight excess of CO_2, and pH = 7.4 $\pm$. It was determined experimentally by running washed CO_2 into flasks of well water containing a little of the proper indicator and some planarians and sealing at different values of pH, that death of the animals from CO_2 does not begin to occur until pH = 5.5 to 5.0 approximately. Susceptibility to CO_2, as to other agents, decreases with advancing age and differs with nutritive and other conditions, so that the minimum lethal concentration of CO_2 differs somewhat for different animals. By adding to the water a little of the proper indicator it is possible to determine the pH at which the animals die in water containing various amounts of oxygen. In this way it has been found that in the low oxygen water

directly from the well they usually die while pH > 7.0, while with the various degrees of aeration used, death almost always occurs before the pH has decreased below 6.0. In other words, even in the aerated water the animals die before they have produced enough CO_2 to kill any of them and in the low oxygen water they die when or before the accumulation of CO_2 has decreased the pH to neutrality. As regards the low oxygen water then there can be no doubt that it is lack of oxygen, not CO_2, that kills, and even in the aerated water lack of oxygen is at least the chief if not the only factor in producing death, although the increasing H ion concentration may alter the amount of difference in susceptibility of the different lots.

In many series, however, a little NaOH has been added to the water in order to avoid all possibility of injurious H ion concentration before the animals are killed by lack of oxygen. With the low oxygen water NaOH is unnecessary but, as noted above, in aerated water, the H ion concentration sometimes approaches the danger point before the animals die from lack of oxygen. The lower limit of lethal concentration of NaOH for the animals is between m/450 and m/500 so that the well water may be made up to any concentration below m/500 with the certainty that the NaOH is not itself lethal. The well water made up to NaOH m/500 has a pH = 9 to 9.5 according to the amount of CO_2 in the water and with lower concentrations of NaOH the pH of course lies between 9.5 and 7.4. The addition of NaOH to the water increases the susceptibility to lack of oxygen even when the concentration is far below the lethal limit, e.g., m/1000 or m/2000, and in general the survival times in water with a given oxygen content decrease as the NaOH concentration increases. In the higher concentrations of NaOH, e.g., m/750 made up in low oxygen water the animals may die from lack of oxygen at pH 8.6 or above and in aerated water at pH 7.0 or above. The use of NaOH in this way makes it possible to bring about death from lack of oxygen at any point within a considerable range of pH on the alkaline side of neutrality.

As might be expected, it is found that the amount of difference in susceptibility of two lots of animals differs with the volume of water used, the oxygen content, the number and size of the animals in the water, the pH at the beginning and the amount of change in pH before death occurs, and some of these factors are mutually related. In no case, however, within the limits indicated, do these factors alter the general susceptibility relations of the lots compared, i.e., the susceptibility of a lot in a particular physiological condition is always greater than

that of another lot in a different condition, in all the modifications of the experimental method described, though the amount of difference may vary to a considerable degree with the different conditions of experiment. Some of these differences will appear in the data presented below, but since the paper is primarily concerned with the effect of starvation on susceptibility to lack of oxygen, the point of chief importance is the increase or decrease in susceptibility with change in nutritive condition, and the degrees of difference with different ranges of H ion concentration and other experimental conditions have to do with other problems. In all cases recorded below where susceptibilities are directly compared, the experimental conditions, except as regards nutrition or physiological age, are either identical or the differences are noted. With very few exceptions only lots in the same tube are directly compared.

Two criteria may be used in determining susceptibility to lack of oxygen; first, the loss of muscular coördination with consequent loss of ability to attach the body to the glass, and the cessation of motor activity which soon follows; second, by the disintegration of the body, both methods giving similar comparative results. As regards both these changes, the body of each individual, except in the more advanced stages of starvation, shows a gradient in susceptibility similar to the gradient in susceptibility to cyanides and other agents, the head region being most susceptible (6). In the later stages of starvation the body is usually almost as susceptible as the head, sometimes more so.

The data on susceptibility are most simply presented in graphic form, and the method adopted for graphing is briefly as follows: since the effects of lack of oxygen, both as regards cessation of movement and disintegration usually begin at the head and progress in an orderly manner along the body, it is possible to distinguish more or less arbitrarily three or four stages in the course of each of these processes. Assigning to each of these stages a numerical value, e.g., stage I, 10; stage II, 20, etc., it is then possible to obtain a numerical value for the average condition of each lot of worms at any given time during the experiment by multiplying the number of worms in each stage by the numerical value for that stage, adding the products and dividing their sum by the number of worms in the lot. These values may then be plotted as ordinates against times as abscissas. By measuring the ordinates downward from a distance above the axis of abscissas equal to the longest possible ordinate, the curves of susceptibility appear as descending curves which reach the axis of abscissas when all worms of the lot have attained the final stage, or by reversing the order of the numerical values

of the stages and measuring upward from the axis of abscissas the same result is obtained. In the following figures the curves are all drawn as descending curves. This method of graphing susceptibility data is described in detail elsewhere (4, p. 81, footnote). With this method the difference in time measured on the axis of abscissas between the upper end and the base of the curve represents the entire time from the beginning to the end of the process of change concerned, either loss of motility or disintegration, and the course of the curve represents in a general way the progress of the change from the head along the body. The less the distance of the curve from the axis of ordinates, the greater the susceptibility, and vice versa. The following graphs are plotted in this way, the time intervals indicated on the axis of abscissas being two hours each and the divisions on the axis of ordinates representing the stages.

It should be noted that these data on susceptibility to lack of oxygen concern ectoderm and body wall and probably the nervous system, but not the alimentary tract. For the loss of motility, changes in cilia, muscles or the nervous system or all three are responsible, but as regards disintegration, oxygen must pass through the body wall to reach the alimentary tract, and when the oxygen content of the water becomes so low that it is not sufficient to maintain the life of ectoderm and body wall, it is probable that very little oxygen is reaching the alimentary tract. As a matter of fact the alimentary tract appears to disintegrate at about the same time as the body wall, but under the conditions of the experiment this fact can have little or no significance.

The standardization of starvation stocks has been discussed in an earlier paper (5), and it need only be said here that the methods of standardization described have been followed with all material used in these experiments, in order that all animals of a given lot should be as nearly as possible alike, not only as regards size and nutritive condition, but as regards the occurrence of fission and regulation.

THE INCREASE IN SUSCEPTIBILITY DURING STARVATION

It may be stated at once that all experiments performed, twenty-two in number, each consisting of two or three lots of from three to five worms each in the same tube or small flask, show that susceptibility of ectoderm and body wall to lack of oxygen increases during starvation, at least up to four months, longer periods not having been used. Since the results are essentially the same in all cases, the differences being

merely differences in the degree of susceptibility, dependent on the conditions of different experiments, it is quite unnecessary to give data or graphs for all series. The following series show the character of the results and some of the differences under different experimental conditions.

Series 708. Figure 1.

Lot I. 3 animals 15 mm. Well fed.

Lot II. 3 animals, originally 15 mm., starved 37 days. At time of experiment 12 to 13 mm.

Lot III. 3 animals, originally 15 mm., starved 99 days, now 7 mm.

These three lots were sealed in a tube in 3 cc. of m/1000 NaOH in well water of low oxygen content.

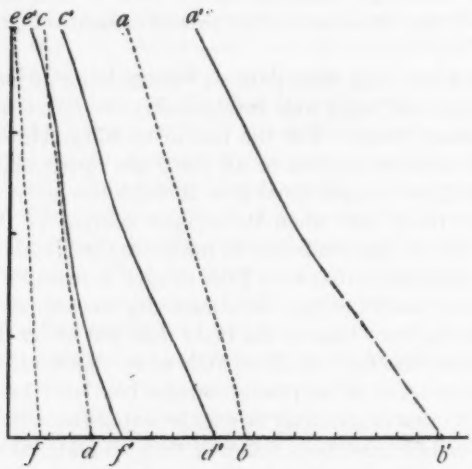

Fig. 1

The curves *ab*, *cd*, *ef* (fig. 1) show the susceptibility to lack of oxygen of lots I, II and III respectively in terms of loss of motor activity and the curves *a'b'*, *c'd'* and *e'f'* show the susceptibility in terms of disintegration. The broken line in the lower part of *ab* indicates that observations were concluded before all animals of this lot were entirely disintegrated.

The fed animals of lot I are least susceptible, those of lot II, starved 37 days, much more susceptible and those of lot III, starved 99 days, most susceptible to lack of oxygen, both as regards loss of motor activity and disintegration. Moreover, the relative susceptibilities of the different lots are very nearly the same in terms of loss of motor activity as in terms of disintegration. This is a perfectly characteristic series. With higher oxygen content, greater volume of water or lower alkalinity the susceptibility curves lie farther to the right and farther apart than in figure 1 but their relative positions are not greatly changed.

Series 614. Figure 2.

Lots I a, II a. Each 3 animals, 25 mm., well fed.

Lots I b, II b. Each 3 animals originally 25 mm.; starved 39 days and underwent fission once during starvation; length at time of experiment 12 to 14 mm.

Lots I a and I b were sealed in one tube in 5 cc. NaOH m/3000 and lots II a and II b in another tube in 5 cc. NaOH m/2000, both slightly aerated.

The curves in figure 2 show the susceptibilities in terms of disintegration as follows: *ab* lot I a; *cd* lot I b; *ef* lot II a; *gh* lot II b. In both pairs of lots the animals starved 39 days are far more susceptible than the well-fed animals.

Since the two pairs of lots were in two different tubes, they are perhaps not strictly comparable, although, except as regards concentration of NaOH, conditions in the two tubes must have been very nearly identical, both containing the

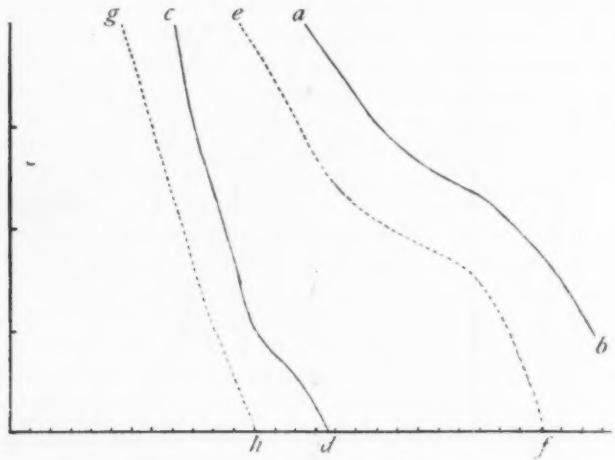

Fig. 2

same number of worms of the same two sizes and the same nutritive conditions in the same volume of water. Figure 2 shows that the susceptibility in NaOH m/2000 (curves *ef*, *gh*) is somewhat greater than in NaOH m/3000. While slight differences in weight, physiological condition, oxygen consumption or oxygen content may exist in the two tubes, the differences in susceptibility indicated by the differences in position of the two pairs of curves, is too great to be the result of such incidental differences and is undoubtedly due, at least in large part, to the difference in concentration of NaOH in the two tubes, the susceptibility being greater in the higher concentration. Other series have given similar results.

Series 715. Figure 3.

Lot I. Posterior halves of animals originally 25 mm. Starved 59 days; underwent regulation to whole animals during early stages of starvation; at time of experiments 8 to 9 mm.

Lot II. Posterior halves of animals originally 25 mm. Starved 121 days; underwent regulation to whole animals during early stages of starvation; at time of experiment 4 to 5 mm.

The two lots of three animals each were placed together in a tube in 2 cc. of low oxygen water made up approximately to NaOH m/500 with as little exposure to air as possible and containing 1/150,000 phenolsulphonephthalein.

Curve *ab* of figure 3 shows the disintegration of lot I, curve *cd* that of lot II, the more advanced stage of starvation being the more susceptible. The high susceptibility of both lots is due to the high concentration of NaOH. This concentration, m/500, was not lethal for animals from the same stocks as the experimental animals in a tube open to air. In this experiment the animals died from lack of oxygen before the pH had fallen below 8.6.

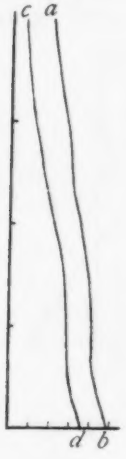

Fig. 3

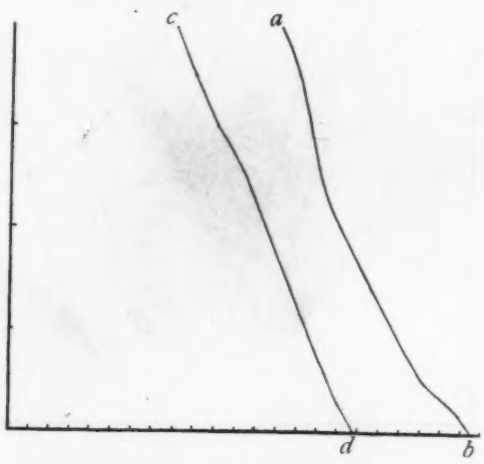

Fig. 4

Series 737. Figure 4.

Lot I. Four animals 16 mm., well fed.

Lot II. Four animals originally 16 mm., starved 18 days; not appreciably reduced in length.

Both lots together in a tube in 7 cc. water containing 1/150,000 phenolsulphonephthalein, but no alkali; water aerated; pH at beginning 7.9.

The curve *ab* in figure 4 shows the disintegration of lot I, the fed animals, curve *cd* that of lot II, the animals starved 18 days. As in other experiments the susceptibility of the starved is greater than that of the fed animals. Since no alkali was added in this series and the water contained considerable oxygen, it became distinctly acid, pH 6.0, before all the animals died. Under these conditions the susceptibility of both lots is less and the difference between the two lots greater than in water with very low oxygen content or with higher alkalinity.

The point of chief interest, however, is that, even with the short starvation period of eighteen days and with no appreciable decrease in size, the susceptibility of the starved animals is distinctly higher than that of the fed animals which represent as nearly as possible the condition at the beginning of starvation.

DIFFERENCES IN SUSCEPTIBILITY TO LACK OF OXYGEN BETWEEN YOUNG AND OLD ANIMALS

It has been shown elsewhere that susceptibility to KNC and rate of CO_2 production decrease in *Planaria* with advancing physiological age and that starving animals show changes in the opposite direction (3), (4), (5), i.e., according to these criteria of physiological age the starving animals become younger. The data of the preceding section show that susceptibility to lack of oxygen increases during starvation and in order to determine whether in this respect also the starving animals undergo changes in the opposite direction from those of progressive development and advancing age, it is necessary to determine what changes in susceptibility to lack of oxygen occur in relation to age in well-fed growing animals.

Comparative determinations of susceptibility to lack of oxygen in animals of different age have been made many times and by different persons in this laboratory, the experiment having been used in laboratory classwork. In these experiments where all animals are well fed, size of animals is used as the most satisfactory criterion of physiological age, for the larger animal has undergone more growth and if a progressive senescence occurs in the species, the larger animal must be physiologically older than the smaller. In all experiments with well-fed animals, the smaller younger individuals are more susceptible to lack of oxygen than the larger and older. Graphs of a few characteristic experiments are given in figure 5.

Series 665.

Lot I. 5 animals, 25 mm. Lot II. 5 animals, 6 to 7 mm.

Both lots from well-fed laboratory stock together in 125 cc. of slightly aerated water. Curve *ab* shows susceptibility of lot I, the old animals, curve *cd* that of lot II, the young animals. The long survival times, 65 hours for lot I and 31 hours for lot II, are due to the large volume of water.

Series 664.

Lot I. 5 animals, 20 to 25 mm. Lot II. 5 animals, 7 mm.

Both lots from well-fed laboratory stock, together in 125 cc. of low oxygen water made up to NaOH m/2000 with as little exposure to air as possible. Curve *ef* shows susceptibility of lot I, curve *gh* that of lot II.

Series 751.

Lot I. 5 animals, 18 mm. Lot II. 5 animals, 7 mm.

Both lots from a stock freshly collected in midwinter and fed only three times in the laboratory. Such worms contain less nutritive reserves than animals which have been a long time in the laboratory. Both lots together in 5 cc. of water made up to NaOH m/750 and slightly aerated. Curve *ij* shows susceptibility of lot I, curve *kl* that of lot II.

Series 754.

Lot I. 5 animals, 20 mm. Lot II. 5 animals, 5 mm.

From same stock as preceding series. Both together in 3 cc. of low oxygen water. Curve *mn* shows susceptibility of lot I, curve *op* that of lot II

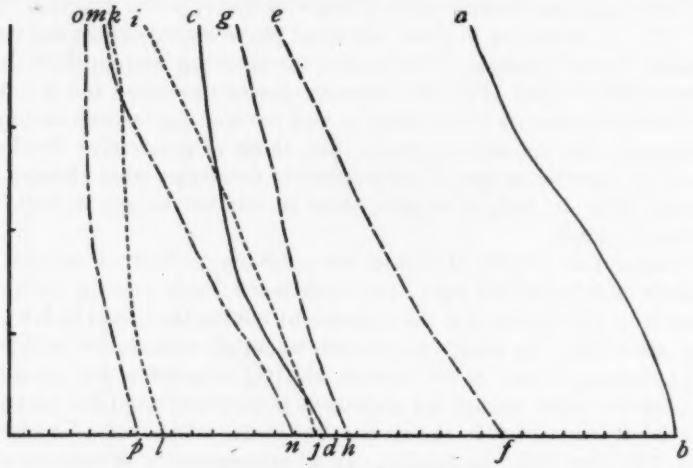

Fig. 5

In these four series the ages compared, the volume, oxygen content and alkalinity of the water differ, consequently only the two curves of each pair are comparable, but in all series the younger animals are distinctly more susceptible to lack of oxygen than the older, though the actual susceptibility and the degree of difference differ with the conditions. There can be no doubt that this difference in susceptibility to lack of oxygen between animals of different size and therefore of different physiological age, indicates a real difference in physiological condition. Moreover, since these data make it clear that susceptibility to lack of oxygen decreases with advancing age and since it was shown in the preceding section that susceptibility to lack of oxygen increases during starvation, it is evident that the changes during starvation are

opposite in direction to those during growth and progressive development, i.e., as regards susceptibility to lack of oxygen, the starving animals become progressively younger.

COMPARISON OF STARVED AND FED ANIMALS OF THE SAME SIZE

The starving animals decrease in size and it is of interest to determine how the rate of increase in susceptibility to lack of oxygen compares with the rate of decrease in size. To obtain evidence on this point the starved, reduced animals are compared with well-fed, growing ani-

Fig. 6

Fig. 7

mals of approximately the same size. Only five experiments of this sort have been performed, but the results are the same in all cases, the starved animals being somewhat more susceptible than the young fed animals. The data of three series are given.

Series 755. Figure 6.

Lot I. 3 animals, starved 42 days. Reduced from 8 mm. to 5 mm.

Lot II. 3 animals from well-fed laboratory stock; 6 mm. (smallest of stock).

Both lots together in 4 cc. of low oxygen water. Curve *ab*, figure 6, shows susceptibility of lot I, curve *cd* that of lot II. Here the fed worms of lot II were slightly larger than the starved animals of lot I.

Series 757. Figure 7.

Lot I. 3 animals, starved 42 days. Reduced from 8 mm. to 5 mm.
Lot II. 3 animals from well-fed laboratory stock; 5 mm.
Both together in 3 cc. low oxygen water. Curve *ab*, lot I; curve *cd*, lot II.
Series 761. Figure 8.
Lot I. 3 animals, starved 79 days. Reduced from 10 to 12 mm. to 4 mm.
Lot II. 3 animals from freshly collected stock fed three times in the laboratory; 4 mm.
Together in 3 cc. low oxygen water; Curve *ab*, lot I; curve *cd*, lot II.

In all three series the higher susceptibility of the starved worms is evident: the differences in the three are dependent upon the differences in size and nutritive condition of the fed, as compared with the starved

Fig. 8

Fig. 9

animals and upon differences in volume and oxygen content of the water, possibly also to some extent upon the stage of starvation (see p. 403).

Although in the earlier work on susceptibility to KNC attention was not particularly directed to this point, it was noted that the susceptibility of starved animals was frequently somewhat higher than that of fed animals of the same size (3, p. 433), and one series is given here.

Series 557 III. Figure 9.
Lot I. 10 worms, starved 81 days. Reduced from 10–12 mm. to 5–6 mm.
Lot II. 10 worms, well-fed; 5 to 6 mm. From freshly collected stock.
Curve *ab*, figure 9, shows the susceptibility of lot I, curve *cd* that of lot II to KNC m/1000.

With the method and scale of graphing, which is the same as that of the preceding figures, the differences in susceptibility appear to be slight, but actually the susceptibility of the starved worms is only about 75 per cent that of the fed animals of the same size. Similar differences have been observed in many other KNC series, but they seem to occur more frequently when the animals were of medium size at the beginning of starvation than when they were very large. It is possible that in the extreme stages of starvation which occur when very large animals are reduced by starvation to small size, the increase in susceptibility does not keep pace with the decrease in size, but further work is necessary to settle this point. It is certainly true that animals after one to three months of starvation are very commonly more susceptible to KNC and in all cases thus far observed are more susceptible to lack of oxygen than fed, growing animals of the same size.

CONCLUSION

The experimental data demonstrate that during starvation susceptibility to lack of oxygen increases, at least in ectoderm and body wall, the method not being suitable for determining conditions in the alimentary tract. This increase in susceptibility parallels very closely the increase in susceptibility to KNC which occurs during starvation. In both KNC and lack of oxygen it is primarily susceptibility of ectoderm and body wall that is determined, though in KNC the susceptibility of the alimentary tract may be roughly determined as differing either more or less from that of ectoderm and body wall (5, p. 253). The data on CO_2 production, however, represent CO_2 production of the alimentary tract as well as that of ectoderm and body wall, and it has been pointed out (5) that the decrease in CO_2 production during the earlier stages of starvation is very largely, if not wholly due to the decrease in metabolic activity of the alimentary tract in the absence of food. In the later stages of starvation even the total CO_2 production increases.

The data on susceptibility to lack of oxygen constitute a further contribution to the problem of starvation. The experiments with animals of different age show that susceptibility to KNC (3), (4) and lack of oxygen and rate of CO_2 production (5) decrease with advancing age, and Doctor Hyman has found that oxygen consumption also decreases during senescence. In short, all the evidence indicates that susceptibility to lack of oxygen in fed, growing animals varies directly with rate of oxidation. In starving animals susceptibility to KNC and to lack of

oxygen increases, CO_2 production also increases, except for the initial decrease noted above, and Doctor Hyman's work shows that oxygen consumption also increases after an initial decrease due to the same factor as the decrease in CO_2 production.

The only logical conclusion on the basis of all the evidence is that the rate of oxidation increases during starvation, in ectoderm and body wall from the beginning and in the alimentary tract in later stages after the initial decrease due to decreased functional activity. If this conclusion is correct, susceptibility to lack of oxygen is, as might be expected, in some degree a measure of rate of oxidation in ectoderm and body wall.

The only other possibility as regards the starving animals is that certain oxidative reactions which require a higher oxygen concentration than those characteristic of fed, growing animals, occur in increasing proportion as starvation advances, but there is at present no evidence in favor of this hypothesis. Apparently the starving planarian oxidizes its own substance with increasing rapidity as starvation advances and the products of metabolism accumulated during growth and progressive development are broken down and removed. The inactive alimentary tract undergoes atrophy and resorption more rapidly than ectoderm, body wall and nervous system and undoubtedly constitutes the chief source of nutrition for the other functionally active organs after the reserves are exhausted. As regards the rate of its fundamental metabolic reactions, the starving animal unquestionably becomes progressively younger, and when it is again fed after a period of starvation, it is in essentially the same physiological condition, as regards susceptibility to KNC (3), (4) and lack of oxygen, rate of CO_2 production (5) and oxygen consumption (Hyman), as well as rate of growth, as a fed, growing animal of the same size. If feeding is continued, such an animal passes again through the progressive stages of the life history of the species from the point at which feeding began. The experimental data on the effect of feeding after starvation have not yet been presented in detail and must be reserved for another paper.

SUMMARY

1. Susceptibility of ectoderm and body wall of *Planaria dorotocephala* to lack of oxygen, as measured either by loss of motility or by disintegration, increases progressively during starvation, up to at least four months.

2. The susceptibility to lack of oxygen of the animal reduced by starvation is about the same as, or slightly higher than that of a fed, growing animal of the same size.

3. The change in susceptibility to lack of oxygen during starvation is in the opposite direction from that which occurs during growth and progressive development in fed animals, and in the light of the facts already at hand concerning CO_2 production, oxygen consumption and susceptibility to KNC, must be considered as evidence of an increase in rate of oxidation during starvation.

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QUANTITATIVE STUDIES ON THE RATE OF RESPIRATORY METABOLISM IN PLANARIA

II. The Rate of Oxygen Consumption during Starvation, Feeding, Growth and Regeneration in Relation to the Method of Susceptibility to Potassium Cyanide as a Measure of Rate of Metabolism

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INTRODUCTION

A review of the literature concerning the rôle of what has been designated "rate of metabolism" in senescence, rejuvenescence and gradients along the body axes in lower animals ((1) to (9) and other references there cited), will show that little or nothing is known which is based on direct quantitative measurements of metabolism in the animals concerned. It has been assumed that relative susceptibility of an organism to the toxic action of potassium cyanide and other substances is a measure of rate of metabolism or rate of oxidation. This assumption has been used extensively as the basis for inferences regarding the metabolism in Planaria and other animals. The only attempts to measure the metabolism in these forms by methods which do not involve assumptions are certain tests reported by Child, (3, pp. 422, 434; 4, pp. 73, 161, 202) of carbon dioxide production by Planaria in Tashiro's "biometer." These estimations were few in number, comparative not strictly quantitative, and we cannot judge from the account how carefully they were controlled. The purpose of the present paper is to present quantitative information regarding the oxidations in Planaria in relation to starvation and feeding, increase in size of the body, and regeneration; and secondly, to compare these results with the reported differences in the susceptibility of Planaria to potassium cyanide.

The term metabolism is commonly used in animal calorimetry to refer to the decomposition of organic compounds (total katabolism)

yielding energy. It may be considered either from the standpoint of the quantities of the different foodstuffs decomposed and the completeness of this decomposition or from the standpoint of the quantities of energy yielded. The latter basis is used for expressing metabolism in a single quantitative term. Lusk, for example, says with regard to the "exact measurement of the metabolism":

> Thus heat may become a measure of the total activity of the body. It is derived from the total metabolism and must be dependent on it and be a measure of it (10, p. 32).

From this standpoint the oxidations are considered important as a source of energy and as a measure of the energy production. The term "rate of metabolism" seems to be used in this sense in some of the references on senescence and axial gradient, especially where studies of the metabolism of man and mammals are quoted (e.g., (4, pp. 63, 65, 271–273, 297–298)). The following statements seem to imply this meaning of the term.

> There can be no doubt that the rate of metabolism or, more specifically, of oxidations in these animals increases during the course of starvation (3, p. 435).
>
> It has been shown that a relation exists between susceptibility and metabolic activity, more specifically the oxidations or energy-freeing reactions (6, p. 50).

It is not certain, however, that the term is always used in the literature concerning "rate of metabolism" as equivalent to the rate of oxidations or of energy production. A distinction seems to be drawn between them in accordance with the following statement:

> While oxidations are fundamental metabolic reactions, and serve in a general way as a measure of metabolic activity, a considerable range of variation in the different reactions which go to make up the metabolic complex may undoubtedly exist (4, p. 72).

A fact which must be kept in mind in using the term metabolism is that while energy transformation in the cell may be large, the corresponding quantity of material transformed may be small. This is true of respiratory metabolism, while on the other hand in a large number of metabolic processes the quantity of material being transformed is relatively very large while the energy change is hardly measurable. As pointed out by Lund (11, p. 168):

It is very doubtful if the rate of oxidations as measured by carbon dioxide production or oxygen consumption can *even approximately*[1] be used as a measure of the rate of total metabolism (either of matter or energy[1]) in a cell, for it has never been shown that the speed of such processes as hydrolyses and changes in colloidal constitution are correlated to the speed of oxygen consumption or carbon dioxide production.

Furthermore, these processes are of a reversible character and matter and energy may pass through them repeatedly before appearing in the external exchanges of the animal. It is evident from these considerations that the terms metabolism and rate of metabolism may be and are used in different senses. On the one hand they may refer strictly to the rate of oxidations or more generally to the rate of energy production as measured by oxygen consumption, carbon dioxide production or heat liberation. On the other hand they may refer in a loose way to the rate of all metabolic processes, involving the complications just mentioned. These two usages are by no means equivalent. It is of vital importance to any profitable discussion of the rate of metabolism in organisms that it shall be clearly understood precisely what is under discussion. This matter cannot be over-emphasized. It is not a question of quibbling over the propriety of one definition as contrasted with another, which is a very unessential matter. But it is extremely important that there shall not be ambiguity as to meaning at any given point, and that an unconscious and unwarranted translation of fact into inference shall not be made. Statements regarding the rate of metabolism may be true when the term means one thing while the same statements may be untrue when the term means something else. If an established fact regarding the rate of metabolism in one sense is used as the basis for an inference regarding the rate of metabolism in a different sense, then great care must be exercised that the inference does not become confused with the fact. The present paper is concerned with the rate of metabolism in the sense of rate of energy production, with the usual qualifications concerning errors in the different methods of direct and indirect calorimetry, and of the possibility of other sources of energy than that represented by the respiratory exchange (see Krogh, (12), chap. i). This statement is made for the purpose of avoiding any ambiguity, and of guarding against the reading into the discussions of more than is intended.

[1] Italics and parenthesis mine.

METHODS

Two species of Planaria, P. maculata and P. agilis,[2] were used in the experiments reported in this paper. The general method for determining the amount of oxygen consumed was to place worms in a bottle of tap water of known content of dissolved oxygen for a stated period of time, and then to remove the worms and analyze the water by the Winkler method for the amount of oxygen remaining. A large carboy was filled with tap water and stirred to make sure that the amount of dissolved oxygen was uniform throughout. The bottles used in an experiment were filled in rapid succession, and the average of the analyses of three or four blanks containing no worms during the experiment was taken to represent the amount of oxygen in each of the experimental bottles at the beginning of the experiment. All bottles were of the same capacity, 132 cc. During analysis the amount of water displaced by the 2 cc. of reagents added is lost. As explained in a previous paper (13, p. 98) removing worms at the end of a test may remove enough water to make it difficult to close bottles after adding the reagents without leaving air bubbles. This can be prevented by adding more reagents or by inserting a glass rod of known volume. In the experiments summarized in tables 3, 4, 5, 6, 8, 12 and 13 the reagents were diluted with an equal volume of distilled water and 2 cc. instead of 1 cc. of each were used. In the other experiments, glass rods of 2 cc. volume were used. The latter method appears preferable although it is unnecessary, if the worms in a single bottle are not too numerous and do not cling too firmly to the glass. The experimental methods are described in more detail in the previous paper. It was shown in that paper (13, table 1) that when 28 bottles were filled with aerated tap water and analyzed at once, the maximum difference between individual analyses did not exceed 0.08 cc. thiosulphate. In the experiments reported previously 15 sets of three or four blanks were analyzed. In no case was the maximum difference between duplicate analyses greater than 0.06 cc. thiosulphate. For the experiments reported in the present paper 37 sets of blanks were analyzed. In one case a difference of 0.12 cc. thiosulphate was noted between duplicate analyses, but this was apparently an error in the reading. In only one other instance did the maximum difference amount to so much as 0.09 cc. thiosulphate. For all the experiments of both papers the average

[2] I am indebted to Dr. Ruth Higley for verifying the identification of these species.

difference beteen the maximum and the minimum analyses in a series of blanks was 0.046 cc. thiosulphate. One drop of thiosulphate from the burette used averages 0.06 cc. in volume. (Titrations were made in a porcelain dish.) These figures therefore indicate the limits of experimental error in the method used and enable one to form a judgment of the accuracy of the data presented in the tables and curves.

The methods of weighing worms have not been described. In the earlier experiments reported in this paper, worms were removed individually to a glass plate in a drop of water, the excess water was absorbed with filter paper and the worm was transferred by means of a probe to a moistened filter paper in a weighing dish. All of the worms which were together in the same bottle in a respiration test were weighed at once, not individually. The error from evaporation was found to average 0.0005 gram. In later experiments a better method was followed. All the individuals from one bottle were transferred in a drop of water to a glass cover slip, the excess water was absorbed with filter paper, and the cover slip was dropped quickly into a weighing bottle half filled with water. When a weighing bottle with a well-ground stopper was used, the error from evaporation was only 0.0002 gram and was much less variable than in the earlier method. These average errors were added to the observed weight in each case.

In order to test the limits of error in the method just described, a single set of worms was weighed repeatedly, as shown in table 1. The worms were weighed three times in rapid succession, and then were allowed to rest undisturbed for a few hours before the next series of weighings. It appeared at once that the first determination in a series was always highest and that the later ones were successively lower. The difference between the first and the third weight was sometimes over 6 per cent. This apparent loss was not permanent, however. After each period of rest the first weight was considerably higher than the last, and almost as high as the first weight of the preceding series. Figure 1 shows these relations graphically. These peculiar results are due, probably, to differences in the amount and character of the mucus. At the moment when excess water is removed from the group of worms on the cover slip, thicker mucus will retain more water; and since care must be exercised not to injure worms by excessive drying, they cannot safely be freed entirely from mucus. Figure 1 shows that the first weights of the different sets make a very regular series decreasing with time and starvation, in which individual variation did not reach 2 per cent. In order to weigh worms under uniform conditions in experi-

ments, all sets were left undisturbed under dark cloth for some time, each set in a separate dish of water, and each set was taken for weighing with as little disturbance to the others as possible.

In experiments extending over a considerable period of time, the worms were weighed at intervals and the weights were plotted on a graph as in figure 2. The weight of each lot of worms at the middle of

TABLE 1

Showing variation among successive weights of a set of Planaria agilis. Five large worms were weighed three times in rapid succession, then were left undisturbed for several hours under dark cloth and were weighed again as before, etc.

DATE	TIME	WEIGHT
July 2	10.50 a.m.	0.0294
	11.06 a.m.	0.0283
	11.24 a.m.	0.0281
July 2	1.30 p.m.	0.0292
	1.46 p.m.	0.0284
	2.02 p.m.	0.0275
July 2	4.44 p.m.	0.0285
	4.58 p.m.	0.0278
	5.12 p.m.	0.0271
July 3	8.41 a.m.	0.0276
	9.04 a.m.	0.0271
	9.17 a.m.	0.0265
July 3	4.28 p.m.	0.0272
	4.40 p.m.	0.0267
	4.52 p.m.	0.0259
July 4	9.19 a.m.	0.0258
	9.34 a.m.	0.0256
	9.48 a.m.	0.0251

the test period was read off from the graph and taken as the average weight during the test. Figure 2 shows the weight curves for the worms of table 7. These worms were undergoing starvation and progressive decrease in size during the experiment. Inspection of the curves shows that the maximum error in individual weights was not over 3 per cent. Inspection of all the weight curves of the experiments in this paper shows that individual variations rarely exceeded 6 per cent.

In the case of figure 2 and table 7, none of the worms had to be discarded because of fission or accidental injury. In some other cases, worms underwent fission during the experiment. Such a worm was removed at once and a new weight was obtained. In later experiments, substitutes were kept ready for replacing such individuals. The substitutes were treated like the worms in the experiment except that their oxygen consumption was not measured unless they took the place of worms in experimental bottles.

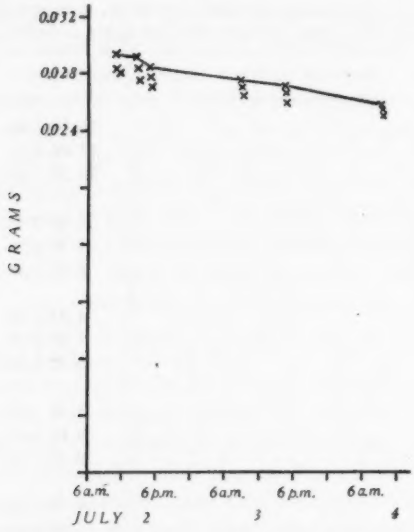

Fig. 1. Showing variation in successive weights of the same set of Planaria agilis. Abscissas, time in 6-hour periods. Ordinates, weight in grams. Solid line connects the first weights of successive series. See table 1.

During the experiments summarized in tables 7, 9 and 14, the respiration bottles were immersed in a water bath within a constant temperature oven at 20°C. Other experiments were carried out at the same temperature in a constant temperature bath.

The test periods were relatively long and the number of worms in each bottle was small. The worms of tables 7, 9 and 14, spent most of the time for six weeks in the respiration bottles. The results represent, therefore, almost continuous measurement of their metabolism through that period, and may be taken as the "normal metabolism" as the term is

used by Krogh (12, p. 116). They do not represent rigidly "standard metabolism" (Krogh, (12), chap. iv) or "basal metabolism" of other authors, because locomotion was not absolutely eliminated, but when Planaria are left undisturbed for several hours they remain at rest for most of the time so that experimental periods of long duration approach nearly to the conditions for "standard metabolism" (see (13), table 9).

In all experiments at least three parallel determinations upon different worms of the same history have been made. Along with the average result, the individual determinations are also given to show the range of variation among them.

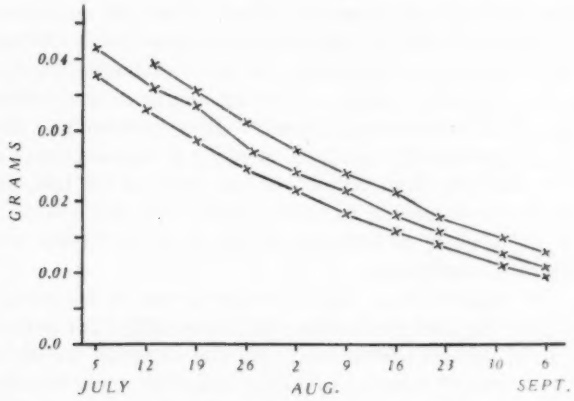

Fig. 2. Curves show decrease in weight of three sets of Planaria agilis of 5 worms each, during 9 weeks of starvation. Abscissas, time in weeks indicated by dates. Ordinates, total weight of 5 worms in grams. See table 7.

OXIDATIONS DURING STARVATION

The rate of metabolism of man and other higher vertebrates falls during the early stages of starvation. Johansson, Landergren, Sonden and Tigerstedt (14) found a decline from 33.15 to 31.20 calories per kilo during the first three days of starvation in man. The heat production during the third to fifth days inclusive remained constant. Among the numerous experiments of Benedict upon *The influence of inanition on metabolism* (15), are three of four days' duration, a fourth of five days' and a fifth of seven days' duration. In general they show a fall in the heat production during these periods, whether it is calculated per unit

of weight or of surface, although in most cases the determinations for the first day were lower than those for the two or even three days following. This fall in heat production during the first few days of starvation is probably due to disappearance of food reserves.

The best observations on man show that metabolism remains fairly constant during prolonged starvation, when calculated per unit of weight or of surface. Lehman, Mueller, Munk, Senator and Zuntz (16) found the respiratory metabolism of Cetti, the professional faster, to remain practically uniform during a fast of 11 days, when calculated per unit of body weight. In Benedict's very complete study of the metabolism in a man starving for 31 days (17), both the respiratory metabolism and the heat liberation, when calculated either per unit of weight or of surface, fell to a practically constant level. Benedict concluded that there was a tendency for the metabolism to divide into three periods: the first period, extending nearly to the middle of the fast, being characterized by a rapidly falling metabolism; the second period, of approximately ten days, showing a comparatively constant metabolism, and the third period, the last week of the fast, showing a general tendency toward an increase (17, pp. 372, 384, 391). The tendency for metabolism to increase during later starvation was slight and of doubtful significance.

Zuntz (18) reports for a dog living for a year in a state of undernutrition, that the heat production, calculated either per unit of weight or surface, fell almost continuously till the eleventh month and rose sharply in the twelfth month toward the end of life. On the other hand Benedict (17, p. 355) quotes experiments by Awrorow on the metabolism of dogs starving for 44 days and 61 days respectively which show a sharp fall in the rate of heat production per unit weight during the first few days, i.e., from about 60 to 48 calories, followed by a gradual rise to a level somewhat above the original, i.e., to 60 to 72 calories, just before a premortal fall.

Warm-blooded animals, however, with their regulatory mechanisms may present special conditions as regards metabolism during starvation. Turning to the cold-blooded animals, the heat liberated by starving frogs per cubic centimeter of frog was found by Hill (19) to decrease during the first two weeks to a practically constant level during the next two weeks. Brunow (20) found a fall in respiratory metabolism in the crayfish during prolonged starvation. A knowledge of the rate of oxidations in Planaria during inanition would add materially to our limited information upon this subject in the cold-blooded animals.

During the first few days of starvation in Planaria, as in other animals, there is a decrease in the rate of oxidations probably due to the diminishing effects of the residual food reserves from the last of the feedings. Table 2 gives a comparison of the rates of oxygen consumption by two lots of worms measured simultaneously, one lot fed daily

TABLE 2

Showing that Planaria agilis after three weeks starvation have a lower rate of oxygen consumption than unstarved worms of the same size. Worms of medium size with short tail because of recent fission; 9 individuals in each bottle. "Well-fed worms" were fed beef liver daily, missing an occasional day, up to the day before the test. "Starved worms" were well fed up to 22 days before the experiment. Temperature 20.4° C. One cubic centimeter thiosulphate equals 0.1711 cc. oxygen at N. T. P. Test period 12 hours. Weighed once within 8 hours after the test

PART	BOTTLE	BLANKS (NO WORMS PRESENT)						
		cc. thio.			*average*			
A	1	4.25			*4.26*			
	2	4.30						
	3	4.24						
	4	4.26						
		I. WELL-FED (STARVED 1 DAY)			II. STARVED 3 WEEKS			
		(1)	(2)	(3)		(4)	(5)	(6)
		Oxygen consumed by the 9 worms in each bottle	Weight of the 9 worms in each bottle	Oxygen consumption per gram per 24 hours	Bottle	Oxygen consumed by the 9 worms in each bottle	Weight of the 9 worms in each bottle	Oxygen consumption per gram per 24 hours
		cc. thio.	*grams*	*cc. oxygen*		*cc. thio.*	*grams*	*cc. oxygen*
B	1	1.17	0.0709	5.6	1	0.73	0.0821	3.0
	2	1.11	0.0723	5.3	2	0.69	0.0784	3.0
	3	0.99	0.0626	5.4	3	0.56	0.0692	2.8
	4	1.03	0.0694	5.1	4	0.68	0.0740	3.1
	5	1.06	0.0636	5.7	5	0.72	0.0755	3.3
	6	1.16	0.0740	5.4	6	0.74	0.0830	3.1
Average		*1.09*	0.0688	*5.4*		*0.69*	0.0770	*3.1*

up to the day before the experiment and the other lot starving for three weeks previous to the test. The worms were of approximately the same weight. The well-fed worms absorbed 1.09 cc. thiosulphate equivalent of oxygen in 12 hours while the worms starved for three weeks absorbed only 0.69 cc. thiosulphate equivalent of oxygen. Cal-

culated per gram of body weight, the well-fed worms consumed 5.4 cc. of oxygen per 24 hours and the worms starved for three weeks only 3.1 cc.

After the first few days of starvation of Planaria, during which the food reserves from the last feedings are effective in increasing the oxidations, the oxygen consumption per unit of weight remains practically constant for a long period of time. Table 3 shows a comparison between Planaria starved for 63 days and others starved for 7 days. The former worms, during the first four days in the laboratory, underwent natural fission, because of their large size and the sudden deprival of food. Only the anterior pieces were used in the experiment. They measured 10 to 13 mm. in length. At the time of the respiration determinations, these were reduced in length to 7 to 8 mm. The second lot of worms starved for only a little over 7 days, were 14 to 19 mm. long at the time of the test. Three successive tests were made. The worms were weighed before the first test and after the last test and the average weight during each period was determined by interpolation, as explained in the section on methods. In each of the three tests the worms after long starvation and those after short starvation absorbed the same amounts of oxygen per gram body weight per 24 hours. While the worms of shorter starvation period were in this laboratory for only six days before the comparisons with those worms which were in the laboratory for 62 days, there was no reason for supposing that they were different from the first lot at the beginning of the starvation period, except that they did not undergo fission. The results of this experiment are confirmed by those of later experiments, tables 4, 5, 6 and 7, in which worms were under continuous observation during the starvation period.

For determining the effects of size, feeding and regeneration upon the oxidations, the rate of oxidation during starvation is the proper standard of reference. All such experiments reported in this paper have been controlled by simultaneous determinations of the oxygen consumption by normal, starving worms, and the continuous observations on these controls give a picture of the course of the oxidations during prolonged starvation. These observations are given in tables 4, 5, 6 and 7. Since the effects of previous feeding were to be avoided in the experiments for which these worms were controls, the worms were starved for several days before beginning observations.

In the experiment recorded in table 4, Planaria maculata that had been starving for several weeks were observed for 34 days during which

TABLE 3

Showing that Planaria agilis after 63 days of starvation have the same rate of oxygen consumption as worms starved for only 7 days. The former worms underwent natural fission 62 to 59 days previous to the first test, because of their large size and sudden removal of food. The anterior pieces from this fission, measuring 10 to 13 mm. in length 59 days before the first test were reduced in length to 7 or 8 mm. at the time of the first test; 10 worms in each bottle. The worms of short starvation period measured 14 to 19 mm. in length; 5 worms in each bottle. The lengths of the three successive tests were, in order, 49, 49 and 46.25 hours. Temperature 20° C. plus or minus 0.15° C., except a temporary fall to 16° C., during the third test, which accounts for the low values in column 10. One cubic centimeter thiosulphate equals 0.1710 cc. oxygen at N. T. P. Worms weighed before the first test and after the third, and the average weight during each period determined by interpolation.

PART	SET	BOTTLE		FIRST TEST			SECOND TEST			THIRD TEST		
				Blanks			Blanks			Blanks		
A	Blanks (no worms present)	1		4.56			4.42			4.29		
		2		4.53		4.54	4.44		4.44	4.25		4.26
		3		4.54			4.45			4.25		
		4		4.54			4.44			4.24		
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
			NUMBER OF WORMS IN EACH BOTTLE	OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	WEIGHT OF THE WORMS IN EACH BOTTLE	OXYGEN CONSUMPTION PER GRAM PER 24 HOURS	OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	WEIGHT OF THE WORMS IN EACH BOTTLE	OXYGEN CONSUMPTION PER GRAM PER 24 HOURS	OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	WEIGHT OF THE WORMS IN EACH BOTTLE	OXYGEN CONSUMPTION PER GRAM PER 24 HOURS
				At end of 7 days starvation			At end of 9 days starvation			At end of 11 days starvation		
				cc. thio.	*grams*	*cc. oxygen*	*cc. thio.*	*grams*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*
B	I	1	5	1.60	0.0318	4.3	1.53	0.0304	4.3	1.13	0.0290	3.6
		2	5	1.68	0.0332	4.4	1.52	0.0311	4.2	1.02*	0.0244*	3.8
		3	5	1.76	0.0399	3.8	1.66	0.0384	3.7	1.41	0.0369	3.5
		Average..		*1.68*	*0.0350*	*4.1*	*1.57*	*0.0333*	*4.1*	*1.19*	*0.0301*	*3.6*
				At end of 63 days starvation			At end of 65 days starvation			At end of 67 days starvation		
	II	1	10	0.57	0.0116	4.2	0.52	0.0110	4.1	0.42	0.0104	3.7
		2	10	0.57	0.0132	3.7	0.62	0.0123	4.4	0.49	0.0115	3.9
		3	10	0.62	0.0126	4.3	0.58	0.0120	4.2	0.46	0.0113	3.7
		Average ..		*0.59*	*0.0125*	*4.1*	*0.57*	*0.0118*	*4.2*	*0.46*	*0.0111*	*3.8*

* 4 worms.

time 12 tests of the oxygen consumption were made, each of about 46 hours' duration. Column 6 shows a steady decline in the average amount of oxygen consumed by the 8 worms in each bottle from 0.105 to 0.037 cc. per 24 hours. In figure 4 the oxygen consumption by the worms during the 34 days of observation, is shown as the control for a

TABLE 4

Showing rate of oxygen consumption by Planaria maculata during starvation. Worms starving for several weeks preceding first test and during the experiment; 8 worms in each bottle. One cubic centimeter thiosulphate equals 0.1766 cc. oxygen at N. T. P. Worms weighed November 20, 27, December 1, 4, 11, 18, 25. Average weight for each test period determined by interpolation. Control for table 8. See figures 4 and 5

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT). AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 8 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	OXYGEN CONSUMED BY THE 8 WORMS IN EACH BOTTLE PER 24 HOURS. AVERAGE	WEIGHT OF THE 8 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS			
							Individual determinations			Average
	hours	*°C.*	*cc. thio.*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
November 20–22....	46.17	19.8–20.3	4.58	1.11	*0.105*	0.0152	5.5	6.1	9.0(?)	*6.9*
November 24–26....	48.00	20.0–20.6	4.47	0.93	*0.085*	0.0134	7.0	5.6	6.2	*6.3*
December 1– 3....	45.92	20.0–20.3	4.55	0.79	*0.075*	0.0108	7.1	8.3	5.9	*7.0*
December 4– 6....	46.15	20.0–20.1	4.29	0.72	*0.068*	0.0099	6.7	8.6	6.0	*6.9*
December 6– 8....	46.13	20.0–20.2	4.02	0.63	*0.060*	0.0093	5.6	7.1	6.5	*6.4*
December 8–10....	46.00	19.8–20.1	4.10	0.64	*0.061*	0.0089	6.5	7.2	6.9	*6.8*
December 11–13....	46.17	20.1–20.3	4.28	0.57	*0.054*	0.0081	6.3	7.2	6.5	*6.7*
December 13–15....	46.25	20.0–20.2	4.21	0.46	*0.044*	0.0075	6.1	5.9	5.4	*5.8*
December 15–17....	46.05	20.0–20.3	4.25	0.58	*0.055*	0.0069	7.5	9.2(?)	7.5	*8.0*
December 18–20....	46.17	20.0–20.3	4.25	0.45	*0.043*	0.0061	6.6	7.5	7.0	*7.0*
December 20–22....	46.05	20.3–18.7	4.31	0.50	*0.048*	0.0058	8.0	9.5(?)	7.6	*8.2*
December 22–24....	51.23	19.9–20.1	4.34	0.43	*0.037*	0.0054	6.3	6.5	7.6	*6.8*

feeding experiment. Column 7, table 4, shows a similar loss of average weight from 0.0152 to 0.0054 gram per 8 worms. Column 11 shows a fairly *uniform rate* of oxygen consumption of 6.5 to 7.0 cc. *per gram weight* during the 34 days of starvation. These last figures are represented graphically in figure 5. Columns 8, 9 and 10 show the varia-

tion in determinations of the individual bottles which indicates the limits of error in the experiment.

Table 5 gives similar observations upon starving Planaria maculata during a period of 27 days. The worms were starved 15 days previous

TABLE 5

Showing rate of oxygen consumption by Planaria maculata during starvation. Worms 10 to 13 mm. in length on January 8; starving for 15 days previous to the first test and during the experiment; 8 worms in each bottle. Weighed January 12, 14, 15, February 5 and 13. One cubic centimeter thiosulphate equals 0.1698 cc. oxygen at N. T. P. See figure 9. For further data on control, see table 12

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT). AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 8 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	WEIGHT OF THE 8 WORMS IN EACH BOTTLE. AVERAGE	OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS			
						Individual determinations			Average
	hours	*°C.*	*cc. thio.*	*cc. thio.*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
January 12–14	41.37	19.9–20.1	4.20	1.20	0.0249	4.5	5.0	4.9	*4.8*
January 15–17	42.66	20.0	4.19	1.20	0.0229	5.2	4.9	5.2	*5.2*
January 18–20	43.23	19.9–20.1 (20.6)*	4.32	1.05	0.0214	4.6	4.6	5.1	*4.7*
January 20–22	46.48	19.9–20.1 (20.3)*	4.66	0.98	0.0205	4.4	4.1	4.5	*4.3*
January 22–24	42.45		4.46	0.81	0.0194	4.1	3.8	4.6	*4.1*
January 24–26	46.90	19.9–20.0 (19.0)*	4.18	0.83	0.0185	4.1	3.8	4.3	*4.0*
January 26–28	45.44		4.30	0.80	0.0175	4.2	3.9	4.5	*4.2*
February 5–9	94.77	16.7–22.6	4.18	1.18	0.0114	4.5	4.4	4.8	*4.6*

* For a short time.

to the experiment. The average rate of oxygen consumption per gram body weight, column 10, was less uniform in this case. The data of column 10 are represented in figure 9, as the control for an experiment in which worms were cut in two and allowed to regenerate. It may be seen that the curves representing the rates of oxygen consumption by

anterior pieces and posterior pieces parallel the irregularities in the curve of the normal worms, indicating some common factor or factors, probably other than the stage of starvation, affecting all worms simultaneously. Table 6 and figure 10 record results of respiration tests on Planaria maculata during 24 days of starvation. The worms were starving for 4 days preceding the experiment. These again were control

TABLE 6

Showing rate of oxygen consumption by Planaria maculata during starvation. Worms 7 to 10 mm. long February 25; starving for 5 days preceding the first test and during the experiment; 10 worms in each bottle. Weighed March 2, 5, 11, 12, 18, 30. One cubic centimeter thiosulphate = 0.1698 cc. oxygen at N. T. P. See figure 10. For further data on controls, see table 13

(1)	(2)	(3)	(4)	(5)	6	(7)	(8)	(9)	(10)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT). AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 10 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	WEIGHT OF THE 10 WORMS IN EACH BOTTLE. AVERAGE	OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS			
						Individual determinations			Average
	hours	*°C.*	*cc. thio.*	*cc. thio.*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
March 2– 4.....	41.40	20.0–20.5	4.59	0.79	0.0160	5.3	5.0	4.8	*5.0*
March 5– 7.....	45.00	20.0–20.2	4.48	0.77	0.0149	4.8	4.8	4.8	*4.8*
March 7– 9.....	47.30	20.0–20.2 (20.5)*	4.54	0.73	0.0136	5.1	4.5	4.9	*4.8*
March 9–11.....	45.65	20.0–20.4	4.56	0.74	0.0123	5.8	5.1	5.5	*5.5*
March 12–14.....	46.38	20.1–20.5	4.65	0.61	0.0106	5.2	5.1	5.4	*5.2*
March 14–16.....	46.16	20.0–20.5	4.70	0.59	0.0096	5.8	5.5	5.4	*5.6*
March 16–18.....	45.00	20.1–20.6	4.68	0.55	0.0087	6.5	5.9	5.4	*5.9*
March 19–21.....	42.90	20.0–	4.62	0.37	0.0076	5.2	5.0	4.1	*4.8*
March 21–29.....	191.09	20.0–20.5	4.47	1.53	0.0062	6.0	5.3	5.1	*5.4*

* For a short time.

data for an experiment in regeneration and again the experimental worms showed individual fluctuations paralleling those of the control.

The experiment recorded in table 7 was the last and most complete series of continuous observations upon starvation. These worms, Planaria agilis, were starved for 7 days before the beginning of the experiment. Three determinations a week were made for six weeks

TABLE 7

Showing uniform rate of oxygen consumption by Planaria agilis during starvation. Worms 16 to 17 mm. long July 5; 5 worms in each bottle; starving for 7 days preceding the first test and during the experiment. July 6 to 19, 1 cc. thiosulphate = 0.1768 cc. oxygen at N. T. P. July 20 to August 16, 1 cc. thiosulphate = 0.1751 cc. oxygen at N. T. P. Worms weighed weekly, namely July 5, 13, 19, 26, August 2, 9, 16, 22, 31 and September 6. See figures 2, 3, 6, 7 and 11. For further data on controls, see table 9

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	BLANKS (NO WORMS PRESENT). AVERAGE OF 4 BOTTLES	OXYGEN CONSUMED BY THE 5 WORMS IN EACH BOTTLE. AVERAGE OF 3 BOTTLES	OXYGEN CONSUMED BY THE 5 WORMS IN EACH BOTTLE PER 24 HOURS. AVERAGE	WEIGHT OF THE 5 WORMS IN EACH BOTTLE. AVERAGE	OXYGEN CONSUMPTION PER GRAM BODY WEIGHT PER 24 HOURS			
							Individual determinations			Average
	hours	°C.	*cc. thio.*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
July 6– 8.....	45.15	22.0–20.0	3.68	1.55	*0.150*	0.0401	3.7	3.8	3.8	*3.7*
July 8–10.....	43.08	20.0–19.6	4.09	1.38	*0.141*	0.0387	3.5	3.6	3.8	*3.6*
July 10–12.....	45.25	20.4–19.9	4.28	1.38	*0.134*	0.0374	3.5	3.5	3.7	*3.6*
July 13–15.....	45.75	20.2–19.4	3.96	1.26	*0.121*	0.0355	3.4	3.4	3.5	*3.4*
July 15–17.....	45.00	20.5–18.8	3.85	1.20	*0.116*	0.0344	3.3	3.3	3.5	*3.4*
July 17–19.....	45.00	20.4–19.2	3.81	1.14	*0.111*	0.0332	3.1	3.3	3.4	*3.3*
July 20–22.....	42.25	19.6–20.0	3.79	1.01	*0.104*	0.0313	3.3	3.2	3.5	*3.3*
July 22–24.....	43.50	20.0–19.7	3.80	1.02	*0.102*	0.0300	3.3	3.4	3.5	*3.4*
July 24–26.....	42.00	19.7–20.0	3.63	0.92	*0.095*	0.0287	3.2	3.2	3.4	*3.3*
July 27–29.....	45.10	19.6–20.2	3.79	0.93	*0.089*	0.0269	3.3	3.3	3.3	*3.3*
July 29–31.....	45.00	19.6–19.8	3.55	0.87	*0.084*	0.0259	3.2	3.3	3.3	*3.2*
July 31–August 2	43.00	19.8–	3.96	0.84	*0.085*	0.0249	3.3	3.4	3.6	*3.4*
August 3– 5....	45.25	19.6–19.9	3.92	0.82	*0.079*	0.0235	3.3	3.3	3.5	*3.3*
August 5– 7....	45.07	19.3–20.0	3.79	0.79	*0.076*	0.0226	3.2	3.3	3.6	*3.4*
August 7– 9....	45.07	20.0–20.7	3.93	0.83	*0.080*	0.0216	3.4	3.7	3.9	*3.7*
August 10–12....	45.42	19.8–19.6	3.73	0.71	*0.067*	0.0204	2.9	3.8	3.4	*3.3*
August 12–14....	45.33		3.84	0.68	*0.065*	0.0196	3.2	3.2	3.5	*3.3*
August 14–16....	45.00	19.6–19.7	4.02	0.66	*0.063*	0.0188	3.3	3.3	3.5	*3.4*
September 8–10	45.27	20.0–	4.44	0.77*	*0.073**	0.0158*	4.3	4.5	4.3	5.1 4.9 *4.6*
September 10–12	45.00	20.4–20.0	4.39	0.71*	*0.068**	0.0151*	4.2	4.5	4.6	5.0 4.8 *4.5*
September 12–14	45.00	19.7–	4.39	0.63*	*0.060**	0.0144*	3.8	3.3	3.7	5.0 4.4 *4.2*

*Ten worms in each bottle.

without interruption. During this time the average consumption per 5 worms per 24 hours decreased from 0.150 to 0.063 cc. oxygen, i.e., to about 40 per cent of the original. The average weight fell, also, during this time from 0.0401 to 0.0188 gram per 5 worms. The fall in oxygen consumption followed very closely, therefore, the loss in weight. This is shown graphically in figure 3 where the average amounts of oxygen consumed are plotted along with the average weights. In general the two curves parallel each other but the curves of oxygen consumption show a greater initial decline, probably representing the remnant of the great fall in oxidations of the earliest stages of starvation due to residual food. When the oxygen consumption per gram weight is calculated, as in table 7, column 11, it is found to be practically constant at about 3.25 to 3.5 cc. after the first week. This is shown graphically in figure 7, where these worms served as the control for an experiment in feeding. The unusual rise on August 7 to 9, figure 7, was thought to be due to disturbing changes in the composition of the tap water. Similar irregularities have been noted in several experiments, under conditions which seem to point to the composition of the tap water as the cause. After an interruption of three weeks, the determinations of oxygen consumption were resumed. Since the worms had become very small, they were redistributed in the bottles and more individuals were added from reserve stock so as to make 10 in each bottle. These reserve worms had the same history as the others. The oxygen consumption per gram weight of body showed a marked rise over previous determinations. It averaged 4.6, 4.5 and 4.2 cc. per 24 hours in three tests. At the same time it was found that worms were beginning to die. They were very susceptible to injury in the manipulations of weighing and analysis. This interfered with continued observation of the rate of oxygen consumption and it was considered that the abnormal conditions of the worms at this time made the observed rise in oxidations of doubtful significance.

Regarding tables 4, 5, 6 and 7, it should be said that they are given in the order in which the experiments were performed. In the last experiment the procedure and manipulations were naturally better so that the results are more uniform. The last experiment was of longer duration, also, and the Planaria agilis, which were used in this case, were found generally more satisfactory for such experiments than Planaria maculata.

From all the above observations on starvation in Planaria, we conclude that the *actual* consumption of oxygen at constant temperature

of 20°C. falls continuously throughout the period of starvation, at first more rapidly, and later more slowly. The body weight also decreases continuously during starvation, figure 3. On the other hand, *the oxygen consumption per gram body weight* (*rate of oxidations*) falls during a short period of the first few days of starvation, due probably to residual food, and then becomes uniform for a long period thereafter. During this long period of uniform rate of oxidations the worms may undergo reduction to one-half or less of their original weight. This

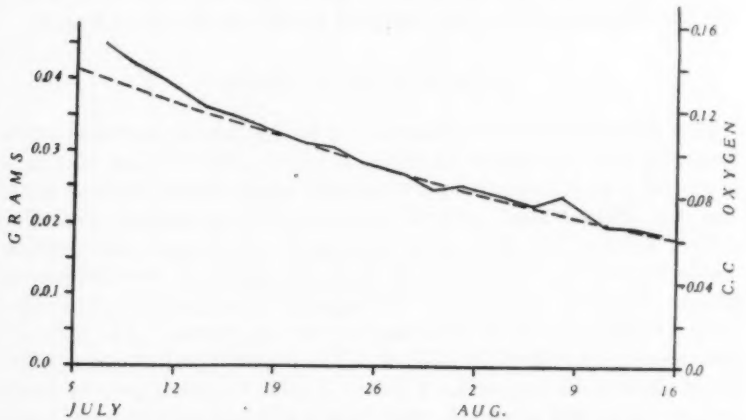

Fig. 3. Curves show decrease in oxygen consumption by Planaria agilis compared with decrease in weight during 6 weeks of starvation. Abscissas, time in weeks indicated by dates. Ordinates at the left, weight of 5 worms in grams. Ordinates at the right, oxygen consumption by 5 worms per 24 hours in cc. oxygen. Broken line represents average weight of three sets of worms weighed once a week. Solid line represents average oxygen consumption by the same three sets of worms, three tests made each week. See table 7 and figure 2.

distinction between the early period of a few days of starvation, during which food reserves from the last feedings are effective in increasing oxygen consumption, and the later period of several weeks, during which the rate of oxidations is uniform, is very important. In the first place, the fact that the rate of oxidations is uniform during a long period of starvation (6 weeks) will serve as an important basis for the study of respiratory metabolism in these forms. In the second place, the fact that food reserves alter the respiratory metabolism for several days at the beginning of starvation makes this period unsuited for such studies.

According to the experiments above and those in the following section on oxidations after feeding, this preliminary period during which food reserves alter the respiratory metabolism may last for a number of days in P. maculata and P. agilis. *In any case before the "basal" or "standard metabolism" has been reached, the rate of oxidations is not a proper basis for comparisons.*

The respiratory metabolism during extreme starvation, when the body has been reduced to a minute fraction of the original weight by starvation alone unaccompanied by fission, has not been studied, and the statements above are not intended to include such later stages.

OXIDATIONS AFTER FEEDING

It is well known that the ingestion of food results in increased oxidations and heat production in higher animals. Planaria can take in at one meal a very large amount of suitable food material, such as boiled egg yolk, clotted blood or beef liver. Several experiments show that such a meal results in a great increase in the oxygen consumption. Table 8 shows an experiment in which P. maculata were fed clotted blood. Columns 4 and 5 give the oxygen consumption of the control worms which were starving throughout the experiment. The control is described in more detail in table 4. The experimental worms were fed blood clots on December 3. Column 7, table 8, shows that the actual amount of oxygen absorbed, which was decreasing progressively before feeding, doubled in the first test following the meal, then declined rapidly, and later more slowly. Figure 4 shows these relations. Column 8, table 8, shows that the worms ingested about 60 per cent of their own weight at this meal. When the rate of oxygen consumption per gram body weight was calculated as in column 12, it was found to rise suddenly from 6.7 to 9.6 cc. oxygen per gram weight and then to fall rapidly to its former level and to remain fairly constant as shown in figure 5. In this calculation the food in the digestive tract is included in the total body weight, thus making the rate of oxygen consumption appear less than its true value. If the weight of food is subtracted from the total body weight for the first test period following the meal, the rate of oxygen absorption per gram of empty body weight becomes 16.5 cc., as shown in table 8, column 12. Upon this basis of calculation, then, the ingestion of food resulted in an immediate increase in the rate of oxygen consumption of over 140 per cent.

TABLE 8

Showing great increase in oxygen consumption by Planaria maculata after feeding clotted blood. Worms not fed for several weeks preceding the first test; 8 worms in each bottle. Experimental worms fed blood clots on December 3. Control worms not fed. Weighed November 20, 27, December 1, 3, 4, 11, 18 and 25. One cubic centimeter thiosulphate = 0.1766 cc. oxygen at N.T.P. See figures 4 and 5. For further data on controls, see table 4.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
			CONTROL WORMS STARVING THROUGH WHOLE EXPERIMENT		EXPERIMENT. WORMS FED BLOOD CLOTS DECEMBER 3						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Oxygen consumed by the 8 worms in each bottle per 24 hours. Average	Oxygen consumption per gram weight per 24 hours. Average	Oxygen consumed by the 8 worms in each bottle. Average	Oxygen consumed by the 8 worms in each bottle per 24 hours. Average	Weight of the 8 worms in each bottle. Average	Oxygen consumption per gram body weight per 24 hours			
								Individual determinations			Average
	hours	*°C.*	*cc. oxygen*	*cc. oxygen*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
November 20–22	46.17	19.8–20.3	0.105	6.9	1.00	*0.095*	*0.0155*	6.1	6.3	5.9	*6.1*
November 24–26	48.00	20.0–20.6	0.085	6.3	0.99	*0.090*	*0.0135*	6.5	7.8	5.9	*6.7*
December 1– 3	45.92	20.0–20.3	0.075	7.0	0.80	*0.076*	*0.0113*	6.8	7.0	6.4	*6.7*
						Experimental worms fed blood clots December 3					
							*0.0101)**	(15.7)*	(15.7)*	(17.7)*	*(16.5)**
December 4– 6	46.15	20.0±0.1	0.068	6.9	1.67	*0.159*	*0.0165*	9.3	9.6	9.8	*9.6*
December 6– 8	46.13	20.0–20.5	0.060	6.4	1.41	*0.134*	*0.0153*	9.0	9.3	8.1	*8.7*
December 8–10	46.00	19.8–20.1	0.061	6.8	1.10	*0.105*	*0.0141*	7.6	7.9	7.0	*7.4*
December 11–13	46.17	20.1–20.3	0.054	6.7	0.91	*0.086*	*0.0126*	6.9	7.4	6.4	*6.8*
December 13–15	46.25	20.0–20.2	0.044	5.8	0.78	*0.074*	*0.0118*	6.1	6.4	6.3	*6.2*
December 15–17	46.05	20.0–20.3	0.055	8.0	0.75	*0.071*	*0.0110*	6.9	6.9	5.9	*6.5*
December 18–20	46.17	20.0–20.3	0.043	7.0	0.66	*0.063*	*0.0099*	6.4	7.4	5.6	*6.3*
December 20–22	46.05	20.3–18.7	0.048	8.2	0.67	*0.064*	*0.0093*	6.8	7.8	6.2	*6.8*
December 22–24	51.23	19.9–20.1	0.037	6.8	0.57	*0.048*	*0.0085*	5.8	6.1	5.4	*5.7*

* Weight of ingested food subtracted from total body weight.

Table 9 gives similar data for an experiment in which P. agilis were fed beef liver. Data for the control worms, which were starving throughout the experiment, are given in columns 4 and 5, and more fully in table 7. After feeding upon liver, July 20, the amount of oxygen absorbed by the experimental worms increased from 0.096 to 0.167 cc. per 5 worms per 24 hours, column 7, table 9. The body weight in-

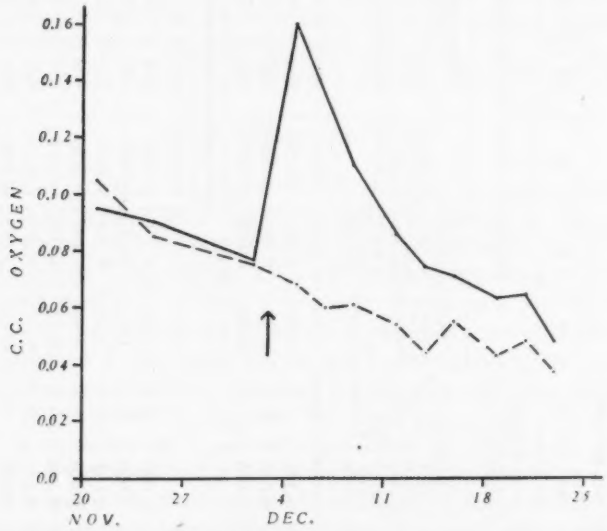

Fig. 4. Curves show decrease in oxygen consumption by starving Planaria maculata, and increase in oxygen consumption after feeding. Abscissas, time in weeks indicated by dates. Ordinates, cc. oxygen consumed by 8 worms in 24 hours. Broken line represents average of three sets of worms, starving throughout the experiment. Solid line represents average of three sets of worms, fed once on December 3. The arrow indicates when the experimental worms were fed. See table 8.

creased from 0.0276 to 0.0353 gram per 5 worms and the rate of oxygen consumption per gram of total body weight, including the ingested food, increased from 3.5 to 4.7 cc., column 12. Deducting the weight of food, the rate of oxygen consumption per gram of empty body weight becomes 6.4 cc. for the first test after feeding, an increase of over 80 per cent. These relations are shown graphically in figures 6 and 7. Inspection of these curves shows that the oxidations rise suddenly with

the taking in of food and decline rapidly during the next week or ten days to a constant level. In figure 6, the curve of the absolute amount of oxygen consumed by the experimental worms ran parallel to the control for two weeks preceding the meal. After feeding, it rose sharply, then declined rapidly during the next 10 to 12 days, and then continued parallel with the control, but at a relatively higher position. This might indicate one of two things, either that the meal had resulted in a certain amount of growth so that the animals which were formerly smaller than the controls became slightly larger, or that the meal re-

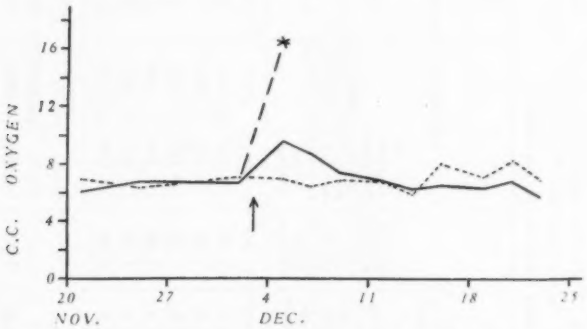

Fig. 5. Curves show practically constant rate of oxygen consumption by Planaria maculata during starvation and increase in rate of oxygen consumption after feeding. Abscissas represent time in weeks indicated by dates. Ordinates represent rate of oxygen consumption in cc. of oxygen per gram total body weight per 24 hours. Broken line represents the average of three sets of worms starving throughout the experiment. Solid line represents the average of three sets of worms fed once on December 3 (time of feeding indicated by the arrow). * represents the rate of oxygen consumption by fed worms in cc. oxygen per gram empty body weight (weight of body if no food had been ingested) per 24 hours. See table 8 and figure 4.

sulted in a permanent increase in the speed of oxidations. Figure 7 and tables 9 and 7 indicate that the former interpretation is correct. A comparison of the average weights given in table 9, column 8, and table 7, column 7, shows that the experimental worms did weigh less than the control worms during the two weeks before the feeding, but that after feeding they did not become reduced again to the weight of the controls. Moreover, figure 7 shows that after August 1 the rate of oxygen consumption by the two lots of worms per gram total body weight ran parallel for 15 days, or till the end of the experiment.

TABLE 9

Showing marked increase in the oxygen consumption by Planaria agilis after feeding beef liver., Worms 15 to 16 mm. long July 5; without food for 7 days preceding the first test; 5 worms in each bottle. Experimental worms fed beef liver July 20. Control worms not fed. Weighed weekly, namely July 5, 8, 12, 19, 20, 26, August 2, 9 and 16. One cubic centimeter thiosulphate = 0.1768 cc. oxygen at N.T.P. from July 6 to 19. One cubic centimeter thiosulphate = 0.1751 cc. oxygen at N. T. P. from July 20 to August 16. See figures 6 and 7. For further data on controls, see table 7.

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
			CONTROL WORMS STARVING THROUGH WHOLE EXPERIMENT		EXPERIMENT. WORMS FED BEEF LIVER JULY 20						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Oxygen consumed by the 5 worms in each bottle per 24 hours. Average	Oxygen consumption per gram weight per 24 hours. Average	Oxygen consumed by the 5 worms in each bottle per 24 hours. Average.	Oxygen consumed by the 5 worms in each bottle per 24 hours. Average	Weight of the 5 worms in each bottle. Average	Oxygen consumption per gram body weight per 24 hours			
								Individual determinations			Average
	hours	°C.	*cc. oxygen*	*cc. oxygen*	*cc. thio.*	*cc. oxygen*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
July 6– 8	45.15	22.0–20.0	0.150	3.7	1.37	*0.133*	*0.0346*	3.9	3.9	3.7	*3.8*
July 8–10	43.08	20.0–19.6	0.141	3.6	1.22	*0.124*	*0.0332*	3.7	3.9	3.7	*3.7*
July 10–12	45.25	20.4–19.9	0.134	3.6	1.24	*0.120*	*0.0317*	3.9	3.8	3.7	*3.8*
July 13–15	45.75	20.2–19.4	0.121	3.4	1.09	*0.105*	*0.0298*	3.6	3.4	3.4	*3.5*
July 15–17	45.00	20.5–18.8	0.116	3.4	1.03	*0.099*	*0.0287*	3.4	3.5	3.5	*3.5*
July 17–19	45.00	20.4–19.2	0.111	3.3	0.99	*0.096*	*0.0276*	3.5	3.4	3.5	*3.5*
						Experimental worms fed beef liver July 20					
							*(0.0259)**	(6.4)*	(6.1)*	(6.7)*	*(6.4)**
July 20–22	42.25	19.6–20.0	0.104	3.3	1.62	*0.167*	*0.0353*	4.8	4.5	4.9	*4.7*
July 22–24	43.50	20.0–19.7	0.102	3.4	1.37	*0.137*	*0.0298*	4.7	4.2	4.9	*4.6*
July 24–26	42.00	19.7–20.0	0.095	3.3	1.15	*0.118*	*0.0289*	4.2	4.0	4.1	*4.1*
July 27–29	45.10	19.6–20.2	0.089	3.3	1.06	*0.102*	*0.0273*	3.8	3.7	3.8	*3.7*

July 29–31	45.00	19.6–19.8	0.084	3.2	0.95	*0.091*	*0.0264*	3.5	3.4	3.4	*3.5*
July 31–August 2	43.00	19.8–	0.085	3.4	0.88	*0.089*	*0.0255*	3.6	3.5	3.4	*3.5*
August 3– 5	45.25	19.6–19.9	0.079	3.3	0.89	*0.085*	*0.0241*	3.4	(lost)	3.4	*3.4*
August 5– 7	45.07	19.3–20.0	0.076	3.4	0.83	*0.080*	*0.0230*	3.6	3.5	3.4	*3.5*
August 7– 9	45.07	20.0–20.7	0.080	3.7	0.86	*0.083*	*0.0220*	3.9	3.6	3.7	*3.8*
August 10–12	45.42	19.8–19.6	0.067	3.3	0.72	*0.068*	*0.0207*	3.4	3.2		*3.3*
August 12–14	45.33		0.065	3.3	0.73	*0.070*	*0.0198*	3.5	3.6		*3.5*
August 14–16	45.00	19.6–19.7	0.063	3.4	0.68	*0.065*	*0.0190*	3.5	3.4		*3.4*

* Weight of ingested food substracted from body weight.

It is evident, therefore, that a single feeding increases the oxidations in Planaria by 80 to 140 per cent and that this increase disappears during the first 10 to 12 days of starvation, the rate of oxidations falling sharply at first and then more slowly to reach a constant level. This result explains why the oxidations in well-fed worms decrease during the first stages of starvation (see expers. 2 and 7 and figs. 3 and 7).

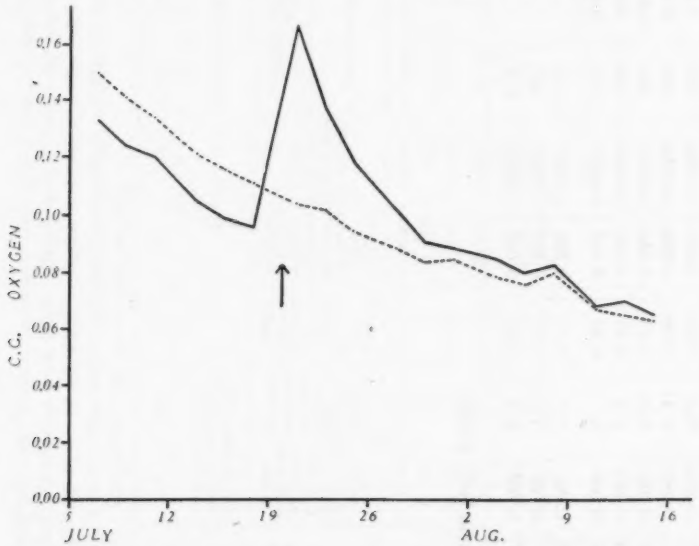

Fig. 6. Curves show decrease in oxygen consumption by Planaria agilis during starvation and increase after feeding. Abscissas represent time in weeks, indicated by dates. Ordinates represent cc. oxygen consumed by 5 worms per 24 hours. Broken line represents the average of three sets of worms starving throughout the experiment. Solid line represents the average of three sets of worms fed once on July 20 (time of feeding indicated by the arrow). See table 9.

OXIDATIONS IN WORMS OF DIFFERENT SIZE

The rate of respiratory metabolism or heat production in higher animals is commonly said to decrease with increasing size and age of the body. It is obvious that a larger animal will use more oxygen, give off more carbon dioxide and liberate more heat than a smaller animal of the same species under the same conditions. In order to make any comparison of the rates of respiratory exchange or heat production in

the two animals, it is necessary to calculate the respiratory metabolism with reference to some common basis such as a unit of weight or surface area. It is understood that such comparisons should be made under standard conditions of *a*, fasting; *b*, uniform temperature; and *c*, muscular rest. But it should be distinctly kept in mind that when animals of different size and age are compared in this way a difference in the rates of respiratory metabolism *may or may not* mean that comparable

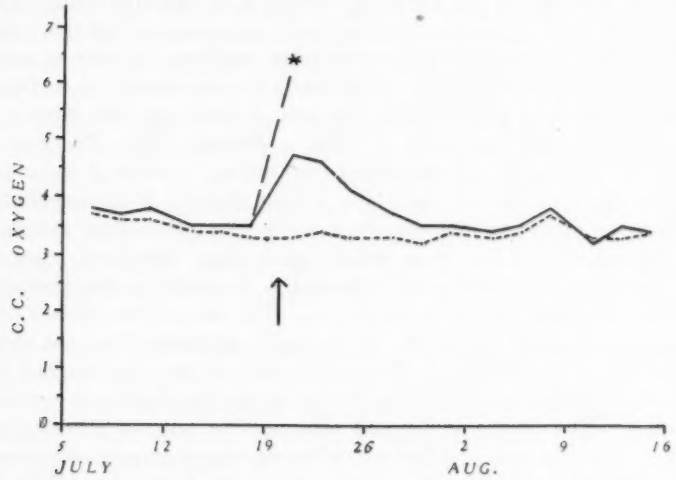

Fig. 7. Curves show constant rate of oxygen consumption by Planaria agilis during starvation and increase in rate of oxygen consumption after feeding. Abscissas represent time in weeks, indicated by dates. Ordinates represent rate of oxygen consumption in cc. oxygen per gram total body weight per 24 hours. Broken line represents the average of three sets of worms starving throughout the experiment. The solid line represents the average of three sets of worms fed once on July 20 (time of feeding indicated by the arrow). * represents the rate of oxygen consumption by the fed worms in cc. oxygen per gram empty body weight (weight of body if no food had been ingested) per 24 hours. See table 9 and figure 6.

cells in the two animals have inherently different rates of oxidation. Different opinions are held regarding the cellular rates of oxidation in these cases (see Benedict, 22). The observation is merely that the total amounts of heat produced are not proportional to the weights or surface areas of the animals.

It is well known that among warm-blood animals the smaller animal produces more heat in proportion to its weight than the larger individual.

Various authors since Rubner (21) have presented evidence that the respiratory metabolism of warm-blooded animals, at least, is proportional to the area of the surface of the body. The respiratory metabolism is often calculated per unit of body surface with the idea of eliminating size differences when other factors are being compared. Why the heat production should be proportional to the area of the skin is not entirely clear. In a general way, the proportionality between the rate of heat production and the area of the radiating surface is of adaptive value to animals which must maintain constant body temperature, but this consideration does not explain what mechanism regulates the rate of energy production in the individual. Apparently it is not simply an accommodation of the heat production to the rate of heat loss (see Krogh (13, pp. 133–140) and Lusk (10, p. 120)). Benedict (22), (23) has objected to the practice of computing metabolism in terms of the unit of surface and has collected data from a large number of normal controls in human metabolism experiments to show that metabolism, calculated per unit of area of the body surface, gives large variations. Some of the variation, may be traced, apparently, to errors in the prevailing method of estimating the surface area. The area of the surface of an animal is not easily measured. It is usually estimated from the weight by means of the formula of Meeh (24) which is based on the law that surfaces of similar solids are proportional to the two-thirds power of their volumes. The bodies of fat and thin persons are not geometrically similar, however, and this formula is known to give large errors in computing the skin areas in such cases. Upon the basis of careful measurements of the areas of the skin of individuals of very different proportions, Du Bois and Du Bois (25), (26), have derived a "linear" and a "height-weight" formula which give the surface area with a maximum error of only 5 per cent in contrast with the average error of 16 per cent and a maximum error of 36 per cent with the Meeh formula. The metabolism seems to be more nearly proportional to the skin area as computed with the new formula than with the older one (27). Among adult mammals, then, the individual of smaller size produces more heat in proportion to its weight than the larger individual, and whatever may be the reason, the amount of the difference seems to be in accordance with the "surface law." This is true of the persons of the same age.

When individuals of different ages are compared, the difference in the rate of heat production per unit of weight does not seem to follow the surface law so closely. The rate of heat production per square meter of surface is higher in youth and lower in old age than in middle life (28),

(29), (27) and (10, p. 128). On the other hand the metabolism of infants per square meter is less than that of older children. That of babies in the first month of life is lowest (12, p. 117); (30), (31), (32), (29, charts 1 to 3). But Bohr's (33) measurements of the respiratory exchanges of pregnant guinea pigs with circulation through the umbilical cord suspended, indicate a rate of oxidations in the embryo per unit of weight not far different from that of the parent. Bohr and Hasselbalch (34) found that the carbon dioxide production by the developing chick embryo from the ninth to the eighteenth days was closely proportional to the weight of the embryo. These observations upon infants and embryos serve to emphasize the fact that the interpretation of differences in rate of respiratory metabolism in warm-blooded animals in relation to size and age, whether calculated per unit of surface or of weight, involves questions of the constitution and functional conditions of the body. While the metabolism studies on warm-blooded animals are commonly cited to show that the rate of oxidations is higher in animals of smaller size and in younger animals, as a matter of fact the warm blooded animals are very unsuited to the critical study of these questions.

Turning to the cold-blooded animals, observations on the rate of respiratory metabolism in relation to size and age are very inadequate. It may be noted at the outset that there is no reason for supposing that metabolism in these animals, of widely different structural organization, should follow the "surface law." It has been shown definitely by Morgulis (36) that this relationship does not exist in the flounder. He finds, rather, that as size of the body increases, the oxygen consumption per unit of body surface increases, and that per unit of body weight decreases, while after removal of the fins the oxygen consumption is proportional to the mass. Buytendijk (quoted by Montuori (35, p. 216)) and Montuori (35, p. 216) both report that Scyllium of smaller size consume more oxygen per kilogram hour than those of larger size.

Observations on the rate of respiratory metabolism in invertebrates in relation to size and age are conflicting but this may be due to unsatisfactory experimental methods. Buytendijk (as quoted by Montuori (35, p. 216)) measured the oxygen consumption by Scorpaena, Octopus and Echinus in relation to body size, and Montuori (35) attempted a survey of these relations in 80 different kinds of marine animals belonging to the coelenterates, echinoderms, worms, crustaceans and fish. In the determinations on some species, the oxygen consump-

tion per unit of weight was greater in the animals of smaller size than in those of larger size. In other series the reverse relationship was recorded, and in still other series the results were highly variable. These results may mean that standard conditions were not maintained in all cases, that the individuals compared were not strictly comparable or that in some cases the forms chosen were unsuited in behavior and respiratory habits to the solution of the question. Each species would require more critical individual study to determine the reasons for the irregularities. The conclusion of Montuori that "the consumption of oxygen, calculated per unit of weight, is, in general, absolutely independent of the dimensions of the animal," hardly seems justified from such highly variable determinations. The subject requires much more careful quantitative work under more definitely controlled conditions than has hitherto been done, before any conclusions of general application can be drawn. Some forms of animals, because of inconstant behavior, are quite unsuited as experimental material.

Careful measurements of the rate of respiratory metabolism per unit of weight in lower cold-blooded animals have indicated, however, that there is a decrease in rate of oxidations as the size of the body increases. Bounhiol (37) found this relationship in the oxygen consumption of annelids of different size, and Miss Wolf, in this laboratory, has found it in the leech, crayfish, branchipus, May-fly nymph and Stonefly nymph, under very conclusive experimental conditions. The number of determinations, made by the writer, of the rate of oxygen consumption by Planaria in various kinds of experiments, makes it certain that consistent results can be obtained for this form with the methods used. Various incidental observations in the course of these experiments point to the fact that smaller worms absorb more oxygen in proportion to their weight than larger worms, and the following systematic experiments show it very clearly.

Table 10 gives comparative measurements of the oxygen consumption by P. agilis of five different sizes. Planaria differ considerably in body proportions, the region posterior to the pharynx being much longer in some individuals than in others. To make worms as nearly uniform as possible, all except the smallest, set I, were cut at the plane of normal fission, a short distance posterior to the mouth. This was done 21 days before the determinations of oxygen consumption were made. All worms were without food for 22 days previous to the experiment. At the time of the respiration test, the worms of set I averaged 6.08 mm. in length, worms of set II, 8.4 mm., those of set III, 11.8,

TABLE 10

Showing decrease in the rate of oxygen consumption by Planaria agilis with increase in size of the body. Worms starving for 22 days preceding the experiment and during the experiment. Tails removed at the fission plane 21 days before the experiment of all sets except set I, the smallest worms. Test period of 24 hours' duration. Temperature 19.6°C. ± 0.01. Capacity of bottles 130 cc. Correction for 4 cc. of water displayed by rods and reagents, multiply by 1.032. One cubic centimeter thiosulphate = 0.1738 cc. oxygen at N. T. P. Worms weighed 6 hours after the test. See figure 8

PART	SET	BOTTLE	BLANKS									
			cc. thio.			*average*						
A	Blanks (no worms present)	1	4.17			4.18						
		2	4.18									
		3	4.20									
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	
			AVERAGE LENGTH OF WORMS	NUMBER OF WORMS IN EACH BOTTLE	OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	OXYGEN CONSUMED PER 10 WORMS PER 24 HOURS		WEIGHT PER 10 WORMS		OXYGEN CONSUMED PER GRAM BODY WEIGHT PER 24 HOURS		
			mm.	*cc. thio.*	*cc. thio.*	*cc. oxygen*	*average*	*grams*	*average*	*cc. oxygen*	*average*	
B	I	1	6.03	20	0.33	0.0295	*0.0349*	0.0069	*0.0070*	4.3	*4.9*	
		2	6.13	20	0.44	0.0394		0.0073		5.4		
		3	6.08	20	0.39	0.0349		0.0069		5.1		
	II	1	8.4	15	0.68	0.0812	*0.0792*	0.0178	*0.0175*	4.6	*4.5*	
		2	8.4	15	0.68	0.0812		0.0179		4.5		
		3	8.4	15	0.63	0.0753		0.0168		4.4		
	III	1	11.7	10	0.89	0.1595	*0.1678*	0.0437	*0.0451*	3.6	*3.7*	
		2	11.7	10	0.97	0.1738		0.0469		3.7		
		3	11.9	10	0.95	0.1702		0.0448		3.8		
	IV	1	14.8	6	0.92	0.2748	*0.2559*	0.0832	*0.0769*	3.3	*3.3*	
		2	14.0	6	0.84	0.2509		0.0772		3.3		
		3	12.9	6	0.81	0.2419		0.0702		3.4		
	V	1	17.8	4	1.04	0.4659	*0.4674*	0.1568	*0.1551*	3.0	*3.0*	
		2	17.9	4	1.08	0.4838		0.1603		3.0		
		3	17.5	4	1.01	0.4525		0.1483		3.0		

those of a set IV, 13.9 mm. and those of set V, 17.7 mm., as shown in column 1. Length, however, is no satisfactory measure of size even though precautions are taken, as described above, to make the worms as nearly comparable as possible in body proportions. The weights, as given in columns 6 and 7, ranged from 0.0070 to 0.1551 gram per 10 worms. The largest weighed twenty-two times as much as the smallest. Because of this great difference in size, the number of individuals in each bottle was varied so as to make the amounts of oxygen absorbed in each bottle nearly equivalent. The average amount of oxygen consumed per gram body weight per 24 hours decreased from 4.9 cc. in the

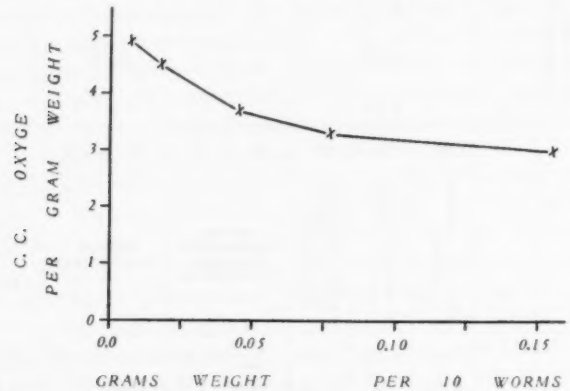

Fig. 8. Curve shows decrease in rate of oxygen consumption by Planaria agilis with increase in size of the body. Abscissas represent weight per 10 worms in grams. Ordinates represent rate of oxygen consumption in cc. oxygen per gram body weight per 24 hours. Each point on the curve represents the average of three sets of worms. See table 10.

smallest worms to 3.0 cc. in the largest. All worms were tested simultaneously under identical conditions. Independent determinations were made for three different bottles of worms of each size, and column 8 shows that, with one exception, there was no overlapping among the individual determinations. Set I, bottle 1, gave a very low value. The titration reading of thiosulphate, column 3, was only 0.33 cc. in this case. It should be remembered that a difference of one drop in titration would make a difference of 0.06 cc., and that titration values as small as this are less accurate than larger ones. This experiment shows, therefore, that the rate of oxidations decreased as the size of the

worms increased. These results are shown in figure 8 where the amount of oxygen consumed per gram body weight per 24 hours at 20°C. is plotted against body weight per 10 worms. This curve shows at a glance that the rate of oxidations decreases with increasing size, and that the decrease in oxidations is not directly proportional to the increase in weight. Among smaller worms, these differences are greater than among larger worms. Most of the difference was between worms less than 0.08 gram weight per 10 individuals. Worms of twice that size showed little further decrease in their oxidations.

It might be thought that the differences in the rates of oxidations among Planaria of different sizes, recorded in table 10, might be due to differences in activity. Was there a graduated difference in activity among the worms of different sizes during the respiration test? This question was tested by removing the heads of worms of different sizes so as to eliminate practically all locomotion. Worms of three different sizes were used, table 11. Those of each size were divided into two sets of three bottles each. A control test was made in which all worms were uncut and normal. Columns 5 and 6 show that, in each case, the two sets of worms of the same size had the same rate of oxygen consumption, and that the differences between the different sizes confirm the previous experiment, table 10. Before the second test, table 11, the heads were removed from the worms in one set of each size. In this test the normal worms, column 10, duplicated the rates of oxygen consumption given in the previous control test, while the headless worms in each case used less oxygen, 85 to 90 per cent of the normal. In comparing the headless worms of the three sizes, however, the size differences remain. The headless small worms used 3.9 cc. of oxygen per gram weight per 24 hours, the medium-sized worms, 3.3 cc., and the large worms, 2.8 cc. We conclude, therefore, that the rate of oxidations in Planaria decreases with increasing total body weight, and that this decrease is not accounted for by difference in locomotion.

The worms of sets II, III and V of table 10 were used as sets II a, III a and V a, respectively, of table 11. Since the worms were weighed for each test, these experiments constitute, in reality, three independent determinations of the normal rate of oxygen consumption by these worms, in relation to size. The three determinations gave the same results. The smaller worms used 4.5, 4.5 and 4.6 cc. of oxygen per gram body weight per 24 hours in the three determinations. The medium-sized worms used 3.7, 3.6 and 3.7 cc. of oxygen, and the larger worms 3.0, 3.1 and 3.1 cc. of oxygen, respectively, in the successive determinations.

TABLE 11

Showing that the difference in rate of oxygen consumption by Planaria agilis of different size is not due to differences in locomotion. Worms starving for 27 days preceding the experiment and during the experiment. Tails removed at the fission plane 26 days before the experiment. Worms of sets II, III and V are of the same sizes as sets II, III and V respectively of table 10. Duration of first test period 24.08 hours, of second test period 24 hours. Worms weighed the day before each test. Just before the beginning of the second test the heads were cut from the worms of sets Ib, IIb, and IIIb. Worms of sets Ia, IIa and IIIa were not cut. Temperature 19.8 C. ± 0.1. One cubic centimeter thisosulphate = 0.1738 cc. oxygen at N. T. P.

PART	SET	BOTTLE			FIRST TEST (CONTROL)				SECOND TEST (EXPERIMENT)				
					Blanks				Blanks				
					cc. thio.		*average*		*cc. thio.*		*average*		
A	Blanks (no worms present)	1			4.35		4.39		4.41		4.44		
		2			4.43				4.48				
		3			4.42				4.43				
		4			4.37				4.43				
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
			WORMS OF SMALL, MEDIUM OR LARGE SIZE	NUMBER OF WORMS IN EACH BOTTLE	OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	WEIGHT OF THE WORMS IN EACH BOTTLE	OXYGEN CONSUMPTION PER GRAM BODY WEIGHT PER 24 HOURS		OXYGEN CONSUMED BY THE WORMS IN EACH BOTTLE	WEIGHT OF THE WORMS IN EACH BOTTLE	OXYGEN CONSUMPTION PER GRAM BODY WEIGHT PER 24 HOURS		WORMS NORMAL OR HEADLESS
					cc., thio.	*grams*	*cc. oxygen*	*average*	*cc. thio.*	*grams*	*cc. oxygen*	*average*	
B	II a	1	Small	15	0.54	0.0195	4.9	4.5	0.51	0.0184	5.0	4.6	Normal
		2		15	0.57	0.0237	4.3		0.53	0.0227	4.2		
		3		15	0.57	0.0233	4.4		0.54	0.0209	4.6		
	II b	1	Small	15	0.53	.0.0209	4.5	*4.8*	0.41	0.0185	4.0	*3.9*	*Headless*
		2		15	0.61	0.0216	5.0		0.43	0.0192	4.0		
		3		15	0.54	0.0196	4.9		0.37	0.0174	3.8		

B	III	a	1	Medium	10	0.82	0.0391	3.7	3.6	0.84	0.0385	3.9	3.7	Normal
			2		10	0.86	0.0423	3.6		0.85	0.0408	3.5		
			3		10	0.82	0.0403	3.6		0.73	0.0353	3.7		
		b	1	Medium	10	0.85	0.0378	4.0	*3.8*	0.64	0.0317	3.6	*3.3*	*Headless*
			2		10	0.93	0.0444	3.7		0.65	0.0377	3.1		
			3		10	0.93	0.0444	3.7		0.68	0.0375	3.2		
	V	a	1	Large	4	0.96	0.0559	3.1	3.1	0.94	0.0537	3.1	3.1	Normal
			2		4	1.01	0.0580	3.1		0.96	0.0561	3.1		
			3		4	0.97	0.0548	3.2		0.92	0.0528	3.1		
		b	1	Large	4	0.95	0.0567	3.0	*3.0*	0.76	0.0513	2.7	*2.8*	*Headless*
			2		4	0.93	0.0556	3.0		0.81	0.0487	3.0		
			3		4	0.94	0.0580	2.9		0.78	0.0526	2.7		

While it is clear that larger Planaria use less oxygen in proportion to their weight than smaller ones, it is not certain that this difference is to be associated with age or senescence of protoplasm. In the case of man, it is commonly supposed that there is a decrease in the rate of oxidations, associated with age, apart from changes in the oxidations, which may be associated with alterations in the size of the body. Such a distinction is not apparent in the data concerning the rate of respiratory metabolism in Planaria. It may be convenient to call a "full-grown" Planarian "old" in comparison with a "half-grown" individual, but it should be remembered that Planaria of the species dealt with in this discussion, after they have attained the more or less variable maximum size for the species, are not known to die of old age. Old age in man is not synonymous with the attainment of a maximum ("adult") body size. We have no evidence, as yet, that a Planarian of constant size becomes senescent or suffers a reduction in the rate of oxidations.

The statement that the rate of oxidations per unit of body weight is higher in small Planaria than in larger ones of the same species, assumes that the worms are compared in the same condition of nutrition, namely that they have been starved long enough to pass the accelerative effects of previous feedings, and that the differences in size are not due to differences in length of starvation. Within the limits of the experiments which have been performed on starvation we have concluded that the rate of oxidations continues practically constant after the early stages. In figure 3, starving Planaria weighed 0.0720 gram per 10 worms on July 12, by which time the accelerative effects of previous feeding had disappeared, and these same worms were reduced in weight to 0.0360 gram per 10 worms on August 16 at the end of the last respiration test. Figure 8 shows that worms of these two weights and of equal starvation differed in oxygen consumption by about 0.5 cc. oxygen per gram body weight per 24 hours. Such a change in the respiratory exchange of the starving worms was not found (see fig. 7). In this case the worms were reduced in weight to one-half of the original. The respiratory exchange in more extreme starvation, with reduction in size to a minute fraction of the original has not been studied.

OXIDATIONS DURING REGENERATION

The species of Planaria employed in these studies reproduce more commonly by agamic fission than by sexual processes. If the rate of oxidations decreases with increasing size of the body, as shown in the

preceding section, then it follows that it should increase with regeneration of small pieces of large worms into complete small worms. Natural fission results in two pieces of very unequal size and character. The anterior piece is much larger and retains the old head and body as far back as a level just posterior to the mouth. After regeneration it is essentially the original parent with a shorter tail but with little change in other proportions. On the other hand, the posterior piece is much smaller and undergoes profound reorganization into an animal with head and body of smaller proportions. Experiments show that the posterior piece undergoes considerable increase in rate of oxidations during regeneration while the anterior piece retains the original rate of oxidations of the parent practically unchanged.

Table 12 records the results of a preliminary experiment in which P. maculata were cut at the normal fission plane and allowed to regenerate, the oxygen consumption of the anterior pieces, the posterior pieces and uncut control worms being followed for 24 days after the operation. Column 4 gives the oxygen consumption by the normal controls (see also table 5). In column 11 the average rate of oxygen consumption by the anterior pieces is seen to be almost equal to that of the normal controls throughout the period of regeneration. The rate of oxygen consumption by the posterior pieces, however, is considerably higher in each determination than that by the controls or that by the anterior pieces. These results are shown also in figure 9.

In a second preliminary experiment, worms were cut just posterior to the auricles, and allowed to regenerate new heads. Under these conditions they were not much reduced in size during the regeneration of the new head. These worms were more comparable, therefore, to the anterior pieces of the preceding experiment than to the posterior pieces. Table 13 shows that the worms regenerating their heads maintained a rate of oxidation *practically equal* to that of control normal worms for the period of observation, 17 days. These results are shown also in figure 10.

Table 14 and figure 11 give a more complete series of determinations of the oxygen consumption by P. agilis during two weeks preceding and four weeks following experimental fission. The rate of oxygen consumption per gram body weight per 24 hours of normal worms ran very uniform throughout the experiment, column 4. These normal worms have been discussed in connection with table 7. The experimental worms duplicated very closely the rate of oxygen consumption by the controls during two weeks preceding the operation, table 14, column 11. After cutting the experimental worms in two at the normal fission plane, the

TABLE 12

Showing increase in the rate of oxygen consumption by Planaria maculata with regeneration. Worms 10 to 13 mm. in length January 8; starving for 15 days previous to the first test and during the experiment; 8 worms in each bottle. January 15 experimental worms cut in two a short distance posterior to the mouth. Control worms not cut. Weighed January 12, 14, 15, February 5 and 13. One cubic centimeter thiosulphate equals 0.1698 cc. oxygen at N. T. P. See figure 9. For further data on controls, see table 5

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
			CONTROL	EXPERIMENT (WORMS CUT IN TWO JANUARY 15, AT NORMAL FISSION PLANE)						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Average oxygen consumption by normal worms per gram weight per 24 hours (cf. table 5)	Part of worm (whole, anterior or posterior)	Oxygen consumed by the worms in each bottle. Average	Average weight of worms in each bottle	Rate of oxygen consumption per gram body weight per 24 hours			
							Individual determinations			Average
	hours	*°C.*	*cc. oxygen*		*cc. thio.*	*gram*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
January 12–14	41.37	19.9–20.1	4.8	Whole	1.13	0.0247	4.7	4.6	4.5	*4.6*
January 15–17	42.66	20.0–	5.2	Anterior	0.71	0.0140	5.0	5.1	5.0	5.0
				Posterior	0.53	0.0077	7.1	7.1	6.5	*6.8*
January 18–20	43.23	19.9–20.1 (20.6)*	4.7	Anterior	0.63	0.0130	4.7	4.9	4.6	4.7
				Posterior	0.45	0.0071	5.8	6.2	6.4	*6.1*
January 20–22	46.48	19.9–20.1 (20.3)*	4.3	Anterior	0.62	0.0125	4.2	4.7	4.4	4.5
				Posterior	0.44	0.0068	6.0	5.7	5.9	*5.8*
January 22–24	42.45		4.1	Anterior	0.49	0.0117	4.1	4.3	4.0	4.1
				Posterior	0.34	0.0064	5.4	5.1	5.3	*5.3*

January 24–26	46.90	19.9–20.0 (19.0)*	4.0	Anterior	0.51	0.0111	4.3	4.1	4.1	4.2
				Posterior	0.36	0.0061	5.6	5.0	5.3	*5.3*
January 26–28	45.44		4.2	Anterior	0.48	0.0104	4.5	4.3	3.9	4.2
				Posterior	0.34	0.0057	5.6	5.6	5.4	*5.5*
February 5–9	94.77	16.7–22.6	4.6	Anterior	0.74	0.0067	5.1	4.9	4.7	4.9
				Posterior	0.48	0.0036	5.7	6.2	5.6	*5.9*

* For a short time.

anterior pieces continued for four weeks to absorb the same amounts of oxygen per gram body weight as the controls, columns 11 and 4. The rate of oxygen absorption by the posterior pieces, however, rose gradually above the controls during regeneration. These posterior pieces reorganized during this period to form complete worms of smaller size than the controls or than the anterior pieces. The following notes indicate the progress of regeneration of the tail pieces.

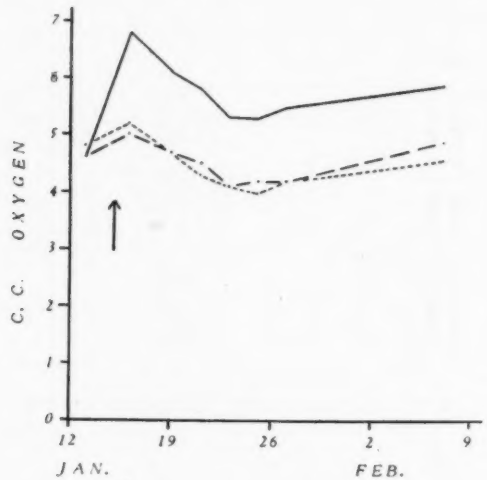

Fig. 9. Curves show increase in the rate of oxygen consumption by Planaria maculata during regeneration of posterior pieces from experimental fission, and no increase, within the limits of error of the experiment, in the rate of oxygen consumption in anterior pieces. Abscissas represent time in weeks, indicated by dates. Ordinates represent the rate of oxygen consumption in cc. oxygen per gram body weight per 24 hours. The broken line represents the control, an average of three sets of normal worms. The solid line represents the average of three sets of posterior pieces from experimental fission. The line of dashes represents the average of three sets of the anterior pieces from experimental fission of the same worms. Worms cut on January 15 (time of the operation indicated by the arrow). See table 12.

July 20. Cut experimental worms across body a short distance posterior to the mouth.

July 24. New head, wedge-shaped. Some locomotion after disturbance of dish.

July 26. Auricles and eyes. Worms elongating.

July 29. Auricles well developed but not full length. Heads appear white to the naked eye. Pigment appearing on head. Inactive till prodded. Locomotion rapid.

August 2. Still white-headed.

August 5. Pigmentation of head still incomplete. Worms more elongated.

TABLE 13

Showing no increase, within the limits of error of the experiment, in the rate of oxygen consumption by Planaria maculata during regeneration of the head. Worms 7 to 10 mm. long February 25; starving for 5 days preceding the first test and during the experiment; 10 worms in each bottle. Heads cut off from the experimental worms just posterior to the auricles March 12. Control worms not cut. Worms weighed March 2, 5, 11, 12, 18 and 30. One cubic centimeter thiosulphate = 0.1698 cc. oxygen at N. T. P. See figure 10. For further data on the controls, see table 6

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
			CONTROL	EXPERIMENT					
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Average oxygen consumption by normal worms per gram weight per 24 hours	Oxygen consumed by the worms in each bottle. Average	Weight of the worms in each bottle. Average	Rate of oxygen consumption per gram body weight per 24 hours			
						Individual determinations			Average
	hours	*°C.*	*cc. oxygen*	*cc. thio.*	*grams*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
March 2- 4	41.40	20.0–20.5	*5.0*	0.77	0.0163	5.0	5.1	4.4	*4.8*
March 5- 7	45.00	20.0–20.2	*4.8*	0.80	0.0149	4.9	5.2	4.8	*5.0*
March 7- 9	47.30	20.0–20.5	*4.8*	0.74	0.0138	4.7	5.1	4.5	*4.8*
March 9–11	45.65	20.0–20.4	*5.5*	0.72	0.0126	5.1	5.3	5.4	*5.3*
						Heads removed from experimental worms on March 12			
March 12–14	46.38	20.1–20.5	*5.2*	0.51	0.0092	4.9	5.4	4.9	*5.0*
March 14–16	46.16	20.0–20.5	*5.6*	0.52	0.0084	5.7	5.8	5.5	*5.6*
March 16–18	45.00	20.1–20.6	*5.9*	0.46	0.0077	5.4	6.1	5.5	*5.6*
March 19–21	42.90	20.0–	*4.8*	0.34	0.0067	5.2	4.9	4.5	*5.0*
March 21–29	191.09	20.0–20.5	*5.4*	1.39	0.0056	5.7	5.9	5.0	*5.5*

The smaller individuals in a population of Planaria usually result from fission and regeneration. This experiment shows that during the processes of regeneration the higher rate of oxidation characteristic of smaller worms is attained. If the respiration of these small worms during growth were followed, undoubtedly a progressive decline in rate

TABLE 14

Showing increase in the rate of oxygen consumption by Planaria agilis with regeneration in the posterior zoöid after artificial fission, and no increase in the rate of oxygen consumption, within the limits of error of the experiment, with regeneration in the anterior zoöid. Worms 17 to 18 mm. long July 5, starving for 7 days previous to the experiment and during the experiment; 5 worms in each bottle before the operation and 10 in each bottle after the operation. Experimental worms cut in two at a level a short distance posterior to the mouth on July 20. Control worms not cut. Worms weighed once each week, namely July 5, 13, 19, 20, 26, August 2, 9 and 16. One cubic centimeter thiosulphate equals 0.1768 cc. oxygen at N.T.P. from July 6 to July 19. One cubic centimeter thiosulphate equals 0.1751 cc. oxygen at N. T. P. from July 20 to August 16. See figure 11. For further data on controls, see table 7

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
			CONTROL	EXPERIMENT						
DATE OF TEST	LENGTH OF TEST PERIOD	TEMPERATURE	Average oxygen consumption by normal worms per gram weight per 24 hours	Part of worm (whole, anterior or posterior)	Oxygen consumed by the worms in each bottle. Average	Average weight of worms in each bottle	Oxygen consumption per gram body weight per 24 hours (rate of oxygen consumption)			
							Individual determinations			Average
	hours	*°C.*	*cc. oxygen*		*cc. thio.*	*gram*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*	*cc. oxygen*
July 6– 8	45.15	22.0–20.0	3.7	Whole	1.68	0.0476	3.4	3.6	3.4	*3.4*
July 8–10	43.08	20.0–19.6	3.6	Whole	1.50	0.0461	3.3	3.3	3.3	*3.3*
July 10–12	45.25	20.4–19.9	3.6	Whole	1.63	0.0456	3.5	3.4	3.4	*3.5*
July 13–15	45.75	20.2–19.4	3.4	Whole	1.48	0.0432	3.2	3.2	3.5	*3.3*
July 15–17	45.00	20.5–18.8	3.4	Whole	1.44	0.0417	3.3	3.3	3.4	*3.4*
July 17–19	45.00	20.4–19.2	3.3	Whole	1.37	0.0402	3.3	3.2	3.4	*3.3*
							Experimental worms cut in two at normal fission plane, July 20.			
July 20–22	42.25	19.6–20.0	3.3	Anterior	1.57	0.0504	3.2	3.2	3.2	3.2
				Posterior	0.49	0.0151	3.3	3.3	3.5	*3.3*

July 22–24	43.50	20.0–19.7	3.4	Anterior	1.54	0.0486	3.1	3.2	3.2	3.2
				Posterior	0.54	0.0142	3.6	3.7	4.2	*3.8*
July 24–26	42.00	19.7–20.0	3.3	Anterior	1.43	0.0468	3.1	3.1	3.3	3.1
				Posterior	0.49	0.0135	3.6	3.7	3.8	*3.7*
July 27–29	45.10	19.6–20.2	3.3	Anterior	1.48	0.0442	3.1	3.2	3.3	3.2
				Posterior	0.49	0.0122	3.8	3.7	3.9	*3.8*
July 29–31	45.00	19.6–19.8	3.2	Anterior	1.40	0.0426	3.1	3.1	3.2	3.2
				Posterior	0.49	0.0118	3.9	3.8	4.2	*4.0*
July 31–August 2	43.00	19.8–	3.4	Anterior	1.32	0.0410	3.2	3.2	3.4	3.3
				Posterior	0.46	0.0113	4.1	4.1	4.1	*4.1*
August 3– 5	45.25	19.6–19.9	3.3	Anterior	1.33	0.0385	3.3	3.3	3.3	3.3
				Posterior	0.48	0.0106	4.3	4.1	4.6	*4.3*
August 5–7	45.07	19.3–20.0	3.4	Anterior	1.29	0.0367	3.4	3.3	3.5	3.4
				Posterior	0.46	0.0101	4.3	3.9	4.8	*4.4*
August 7– 9	45.07	20.0–20.7	3.7	Anterior	1.33	0.0350	3.5	3.5	3.8	3.6
				Posterior	0.49	0.0097	4.8	4.7	5.2	*4.9*
August 10–12	45.42	19.8–19.6	3.3	Anterior	1.17	0.0329	3.3	3.4	3.5	3.4
				Posterior	0.41	0.0090	4.4	4.2	4.4	*4.4*
August 12–14	45.33		3.3	Anterior	1.15	0.0317	3.3	3.6	3.6	3.5
				Posterior	0.38	0.0086	4.4	3.7	4.6	*4.2*
August 14–16	45.00	19.6–19.7	3.4	Anterior	1.08	0.0304	3.3	3.4	3.6	3.4
				Posterior	0.37	0.0082	4.3	4.1	4.6	*4.3*

of oxygen consumption would be found as the animals became larger. It is not very convenient to follow the oxidations in the same worms during growth, but it seems unnecessary to do so. Worms of different sizes selected from a laboratory stock of the same history, except as regards reproduction, and tested simultaneously under identical conditions, give more certain results. Such experiments are described in the preceding section.

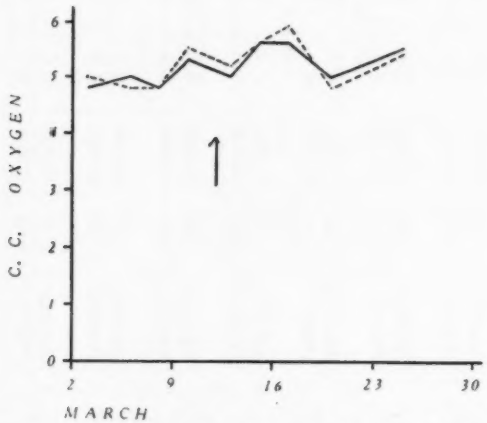

Fig. 10. Curves show no increase, within the limits of error of the experiment, in the rate of oxygen consumption by Planaria maculata during regeneration of the head. Abscissas represent time in weeks, indicated by dates. Ordinates represent rate of oxygen consumption in cc. oxygen per gram total body weight per 24 hours. The broken line represents the control, an average of three sets of normal worms. The solid line represents the average of three sets of worms whose heads were cut off just posterior to the auricles on March 12 (time of the operation indicated by the arrow). See table 13.

It should be clearly understood that these experiments upon regeneration of Planaria are not advanced as evidence that the regeneration process, as such, or, more generally, that growth or morphogenesis, necessitates a higher rate of oxidation. In figure 11 the rate of oxygen consumption in the tails rose progressively during regeneration and *did not show a fall* with completion of the process. The higher rate at the end of the process is attributed to the new organization of the worm, i.e., to the result of the process, not to the process itself. At least the experimental data do not constitute a secure foundation for concluding otherwise.

SUSCEPTIBILITY AS A MEASURE OF OXIDATIONS

The susceptibility of Planaria and other animals to the toxic action of alcohol, potassium cyanide and other substances, in other words, the length of time the animals live in solutions of these substances, has been used extensively by Child, Hyman and others as a measure of the "rate of metabolism." This practice has been based upon inadequate knowledge either of the nature of the action of these toxic agents

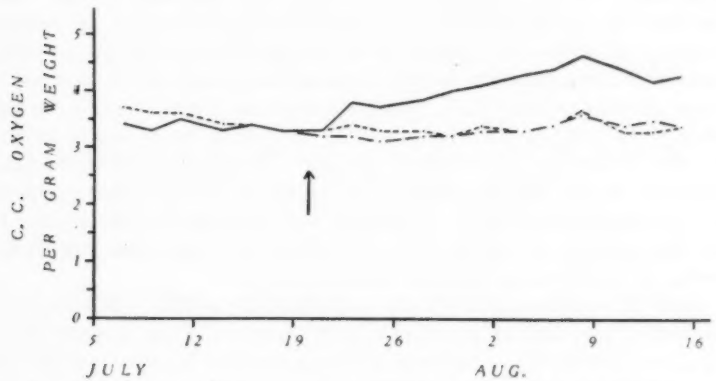

Fig. 11. Curves show increase in the rate of oxygen consumption by Planaria agilis during regeneration of posterior pieces from experimental fission, and no increase, within the limits of error of the experiment, in the rate of oxygen consumption in the anterior pieces. Abscissas represent time in weeks, indicated by dates. Ordinates represent rate of oxygen consumption in cc. oxygen per gram total body weight per 24 hours. The broken line represents the control, the average of three sets of normal worms. The solid line represents the average of three sets of experimental worms before the cutting operation on July 20, and of the posterior pieces after that date. The line of dashes represents the average of the three sets of anterior pieces from the operation. The time of the operation is indicated by the arrow. See table 14.

or of the parallelism between the susceptibility of animals and their "rate of metabolism." The substances first used for this purpose were anesthetics and potassium cyanide, sometimes classed as an anesthetic. It was supposed that these substances inhibit the oxidations, and they were spoken of as "depressing agents" (1, p. 571); (2, p. 150); (3, pp. 419, 420, 444); (4, p. 65); (8, p. 104). The effect of none of these substances upon the oxidations in Planaria or other animals upon which

they were used was determined. The writer (13) has shown that potassium cyanide, the agent most used for the susceptibility studies, does inhibit oxygen consumption in Planaria, but it does not follow from this fact that the worms with higher rate of oxidation must be more susceptible to the cyanide, nor that those that are more susceptible to cyanide must have a higher rate of oxidations. Furthermore, many other substances of very different chemical nature and probably of very different physiological action have been used for determining susceptibility. Before drawing conclusions regarding metabolism from the data of susceptibility, in the absence of knowledge of the nature of the action of the toxic agents used, it should be determined, at least, that there is a parallelism between susceptibility and rate of oxidations under all normal conditions. This has not been done excepting for the comparisons with the carbon dioxide production by Planaria in the Tashiro "biometer" previously mentioned. Upon the basis of the data presented in the present paper, it is possible to make certain comparisons between susceptibility of Planaria and their rate of oxidations. If the susceptibility is a measure of the oxidations, it must not vary independently of the rate of oxygen consumption.

Table 15 summarizes the facts regarding susceptibility and the rate of oxygen consumption per unit of body weight. Four normal conditions are considered; *a*, increase in the size of the body by growth; *b*, decrease in the size of the body by agamic reproduction; *c*, starvation; and *d*, feeding. Starvation is naturally divided into the early and the later stages, making five items in the table. In the so-called "indirect susceptibility method," less susceptibility, or, conversely, greater *resistance*, is supposed to indicate a higher "rate of metabolism," while in the "direct method" greater *susceptibility* is supposed to indicate a higher rate of metabolism or rate of oxidations. The letters in the table refer to the following quotations and references to the literature on susceptibility:

A. (1) pages 544 to 547. Figures 1 and 2.

(4) page 83 and figure 4.

B. (1) pages 555 to 564.

C. "In this connection it should be noted, again, that when well-nourished worms are kept without food, the resistance increases somewhat during the first three or four weeks, as the reserves disappear and after that decreases rapidly" (1, p. 549).

"If the resistance of the worms to anesthetics is in any degree a criterion of age, as it seems to be, then it is evident that while starvation alone does not

TABLE 15

Showing that susceptibility of Planaria to the toxic action of alcohol or potassium cyanide varies independently of the rate of oxygen consumption. Changes in susceptibility determined by Child. For references, see text pages 464 to 466 under letters A, B, C, etc., as given in table. Changes in rate of oxygen consumption determined by the writer

	(1)	(2)	(3)	(4)
	CONDITION OF PLANARIA	SUSCEPTIBILITY TO ALCOHOL AND POTASSIUM CYANIDE		RATE OF OXYGEN CONSUMPTION
		Resistance in "indirect method"	Susceptibility in "direct method"	
I	Increase in size by growth	Decreases A (⇔ decreased oxidations)	Decreases F (⇔ decreased oxidations)	Decreases
II	Decrease in size by regeneration of posterior piece of large worm	Increases B (⇔ increased oxidations)	Increases G (⇔ increased oxidations)	Increases
III	Starvation early stages (5–15 days)	*Increases* C (⇔ increased oxidations)	*Increases* H (⇔ increased oxidations)	*Decreases*
IV	Starvation later stages (5–8 weeks)	Increases D (⇔ increased oxidations) then *Decreases* (⇔ decreased oxidations)	*Increases* I (⇔ increased oxidations)	*Constant*
V	Feeding	*Increases* E after 48 hours (⇔ increased oxidations)	*Decreases* J (⇔ decreased oxidations)	*Increases* within 48 hours

rejuvenate these animals, *except to a slight extent in the early stages*,[3] yet feeding after a long period of starvation does accomplish this very effectively" (1, p. 555).

"During the first two or three weeks of starvation before any marked reduction occurs, the rate of metabolism increases but decreases after reduction begins (indirect method, meaning that resistance increases during first two or three weeks) (3, p. 437).

[3] Italics mine.

D. "I have performed numerous experiments with worms in various stages of starvation and always with the same result, viz., the reduced worms show even less resistance than before reduction" (1, p. 547).

E. (1) page 555, quoted above under C.

"The effect of feeding after starvation does not appear as increased resistance to any marked extent until at least 48 hours after feeding. Probably this period of time represents that necessary for the attainment of a certain stage and rate of the metabolic reactions after feeding" (1, p. 549).

F. (3) pages 423 to 427.

(4) pages 93 to 100.

G. (4) pages 105 to 110.

H. "Animals which have been without food for only a few days are distinctly more susceptible to depressing agents than animals which have been fed up to the beginning of the experiment" (3, p. 434).

(4) pages 156 to 160, especially table 11, page 157.

I. "In all several hundred individuals have been testd and always with the same result, viz., increase in susceptibility from the beginning of starvation on until the animals are reduced to a minute fraction of their original size and death occurs. If the method is valid this increase in susceptibility must mean that the rate of the metabolic processes increases as starvation and reduction proceed, a conclusion exactly the opposite of that reached from my earlier work with the indirect method alone" (3, p. 427).

(4) pages 156 to 160, especially table 11, page 157.

"But the results obtained in later investigations by the direct susceptibility method which have been briefly presented above, and the confirmation of these by the estimates of carbon dioxide production, force us to the conclusion that the rate of metabolism increases during starvation. This being the case, the decrease in capacity for acclimation in starved animals cannot be due to a low rate of metabolism, but must be associated with the nutritive condition in some way independent of the metabolic rate" (4, p. 165).

J. "Each feeding is followed by a distinct decrease in susceptibility, and later, as the animals begin to starve, the susceptibility increases again" (4, p. 169).

Inspection of this table shows at a glance in what cases susceptibility and rate of oxygen consumption per unit of body weight agree and in what cases they disagree. When the size of the body is increased by growth, part I, the resistance by the indirect method decreases, the susceptibility by the direct method decreases and the rate of oxygen consumption decreases. When the size of the body is decreased by experimental fission and regeneration, part II, these three items all increase. These facts are consistent with the theory of a relationship between susceptibility and rate of metabolism. But when the conditions of starvation and feeding are considered, the picture changes. During the first stages of starvaton the rate of oxygen consumption falls sharply, column 4, but the susceptibility by the direct method increases instead of decreases. This striking decrease in the oxidations occurs chiefly

during the first few days. Since the indirect method requires a number of days for making determinations, it is perhaps not well adapted for catching the conditions during this brief period. Statements were made, however, that the resistance by this method increased somewhat during the first three or four weeks without food, as contrasted with the decrease afterwards. During the later stages of starvation, i.e., after the first 10 days, approximately, the rate of oxygen consumption runs practically constant, while the susceptibility in the direct method continues to increase steadily. In the indirect method, the increase in resistance which begins in the early stages is reported to continue during a total of three or four weeks, after which it is followed by a decrease in resistance. It should be clearly understood that this table and discussion refer only to the first seven to nine weeks of starvation, and do not include later stages of more extreme starvation. What changes may occur in the respiratory metabolism, later on, do not concern the point under discussion, which is that the susceptibility of Planaria to potassium cyanide and alcohol, and the rate of respiratory metabolism per unit of total body weight vary independently of each other. Such independent variations are clearly shown within the periods which are considered in this discussion. The fall in rate of oxidations during the early stages of starvation is *large* and is not reflected in any decrease in susceptibility. On the other hand, the increase in susceptibility *during the period of starvation included in these experiments is large* (4, p. 157, table 11) and is not paralleled by an increase in rate of oxygen consumption. It may be noted, furthermore, that the discrepancies revealed in the table are too great to be altered materially by minor differences in the respiratory metabolism of different species of Planaria.

Feeding causes an enormous increase in the rate of oxygen consumption, but it is reported (4, p. 169) that "each feeding is followed by a distinct decrease in susceptibility, and later, as the animals begin to starve, the susceptibility increases again" (direct susceptibility method). In the indirect method, feeding results in an increased resistance as well as in an increased oxygen consumption but it may be noted that "the effect of feeding after starvation does not appear as increased resistance to any marked extent until at least 48 hours after feeding." The maximum effect of feeding in increasing the rate of oxygen consumption is within this 48-hour period. These comparisons show, therefore, that susceptibility in Planaria varies independently of rate of respiratory metabolism.

B. L. Lund (38) and E. J. Lund (11), (39) have shown a similar lack of agreement between susceptibility and rate of oxidations in Paramecium. Starving Paramecia are more susceptible to potassium cyanide than those that are well fed, but the starving Paramecia consume less oxygen and give off less carbon dioxide. Child (7, p. 217) has criticized Lund because "he has apparently failed to recognize an essential point, viz., that susceptibility as measured by progress of death and disintegration concerns primarily the ectoplasm or the body-surface and body-wall. Statements to this effect have been made repeatedly in the work on susceptibility." These statements, however, give no indication that the "*rate of metabolism*" *under discussion* "concerns primarily the ectoplasm or the body-surface and the body-wall." On the contrary, statements have been made repeatedly in the work on susceptibility to the effect that estimations of the carbon dioxide production by Planaria in the Tashiro "biometer" have confirmed results obtained by the susceptibility method (e.g., (3, pp. 422, 343); (4, pp. 73, 161, 202); (8, p. 104)). The following quotation may suffice:

> The rate of production of carbon dioxide in the starved, reduced animals is practically equal to that in the young, growing animal of the same size, and this rate is much higher per unit of body weight than that in large, old animals. The results obtained by the direct susceptibility method are thus fully confirmed by the estimations of the carbon dioxide production (in the "biometer") (4, p. 161).

But they are not confirmed by measurements of the oxygen consumption by Planaria made by the writer, nor by measurements of the oxygen consumption and carbon dioxide production by Paramecium made by Lund.

CONCLUSIONS

In conclusion it should be noted that the purpose of these studies is to contribute to the critical analysis of the rôle of rate of respiratory metabolism in the animal organism. It may be conceived to be the function of bold and suggestive theories such as those of Child regarding the "rate of metabolism" to serve as a basis for analysis of all the factors involved in the question. All facts should be welcome and should be examined with critical impartiality to place them in their proper relation to the general problem. As the analysis proceeds, new aspects appear and new need for discrimination between the various factors involved. The term "rate of metabolism" may be used in different senses. Attention has been confined in this paper to the rate of respiratory metabolism. Even this term is capable of meaning different things. What should seem to be the only strictly sound basis for

comparisons of the relative rates of respiratory metabolism in animals of different size and age are data that give us the rate of respiratory metabolism per unit of weight or volume of *living protoplasm* in the body of the animal. No other basis is really satisfactory. Material should be selected and methods devised which supply these conditions as nearly as possible. Warm-blooded animals are very unsatisfactory material for such work, and this fact should be considered before drawing conclusions of general application from the data of mammalian calorimetry. If the method of susceptibility to the toxic action of such substances as potassium cyanide, when applied to the lower invertebrates, could provide a means of approaching the ideal conditions in measuring the "rate of metabolism" or rate of oxidations, it would be of very great value in the analysis of these problems. This method has been compared in the present paper with measurements of the rate of oxygen consumption in proportion to the weight of the body, and it has been found that the susceptibility does not give a reliable index of the rate of oxidations as thus defined. Since we have no data regarding the rate of oxidations upon the basis of any other definition, we must conclude that, in the present state of our knowledge regarding the action of these substances, only direct methods are reliable for determining the rate of respiratory metabolism.

SUMMARY

1. The oxygen consumption by P. maculata and P. agilis decreases progressively during starvation at constant temperature, more rapidly at first and somewhat more slowly later. The body weight also decreases during starvation, and the worms become smaller in body dimensions.

2. The rate of oxygen consumption per unit of body weight in starving P. maculata and P. agilis decreases rapidly during the first few days, due to the decreasing accelerative effect of food residuum left from the previous feeding. The length of this period may vary with the temperature and the amounts and kinds of food previously ingested, but in the experiments reported it lasted as long as 10 to 14 days, although the accelerative effects were small after the first 7 days.

3. At the end of this period of the accelerative effects of food reserve from previous meals, the rate of oxidations reaches a constant level which is maintained for several weeks in P. agilis (5 to 8 weeks in the experiments reported). During this period of constant rate of oxidations, the starvation results in a decrease in the body weight of at least one-half of the original.

4. The ingestion of food by starving P. maculata and P. agilis results in a great increase in the oxygen consumption (80 to 140 per cent in the experiments reported). After this initial rapid rise, which reaches its maximum within the first 48 hours or less, the *rate of oxidations per unit of body weight* falls more slowly during 7 to 14 days to reach a constant level. When this constant level is reached after a single feeding, the rate of oxygen consumption per unit of body weight is the same, within the limits of error of the experiment, as it would have been if the animal had not been fed. However, after the rate of oxidations has returned to this constant level the animal weighs more and consumes more oxygen in absolute units than would have been the case if the animal had not been given a meal (see figs. 4 and 6).

5. The later period of starvation, after the accelerative effects of food reserves have disappeared, is the proper basis of reference in studying respiratory metabolism in Planaria.

6. Larger Planaria have a lower rate of oxidations per unit of total body weight than smaller worms of the same species when the latter do not owe their small size to starvation. The experiments reported do not permit making any general statement to cover all cases of differences in size due to starvation alone since the more extreme stages of starvation are not included in this study (see paragraph 3 above).

7. After fission of a large worm, the tail piece, which becomes reorganized into a small-sized worm, gains a higher rate of oxygen consumption per unit of body weight. With regeneration in the anterior piece, which does not involve important alterations in the body, the rate of oxygen consumption remains constant within the limits of accuracy of the method. It is not assumed that the increase in rate of oxidations in the posterior piece is due to the use of measurable amounts of energy in the processes of regeneration or morphogenesis as such.

8. The susceptibility of Planaria to the toxic action of potassium cyanide and alcohol, as reported by Child, varies independently of the rate of oxidations per unit of body weight, and is, therefore, not a reliable measure of the oxidations as thus defined. If the rate of oxidations or the "rate of metabolism" is defined differently, then it remains to be shown that susceptibility is a reliable measure of either.

APPENDIX

Since the above was written, three papers have appeared which deal with the respiratory metabolism in Planaria (40), (41) and (42). Hyman (41) has attempted to strengthen the theory that susceptibility is a

measure of rate of oxidations by showing that potassium cyanide inhibits the oxidations in Planaria dorotocephala; her results confirm those of the writer reported in the first paper of this series (13). The two studies supplement each other since the experiments of Hyman covered short periods of time while those of the writer covered longer periods. Hyman argues that "since cyanides decrease the rate of oxygen consumption, it follows that cells or organisms or parts of organisms which have the highest rate of respiratory exchange will be more susceptible to cyanide and will, therefore, die in lethal concentrations of cyanide faster than parts or organisms respiring less actively" (41, p. 353). The writer does not wish to discuss this argument further than to call attention to the facts observed in Paramecium and Planaria, namely, that fed animals have a higher rate of respiratory exchange but live longer in lethal concentrations of cyanides than starving animals.

Child (40) has compared the rates of carbon dioxide production by equal weights of Planaria dorotocephala of different sizes and in different stages of starvation, as determined by the phenolsulphonephthalein indicator method. The data do not permit a calculation of the actual quantities of carbon dioxide produced, but show that the worms in one tube caused a higher or lower hydrogen ion concentration than those in another tube. Child concludes from his experiments with this indicator method that the rate of carbon dioxide production is greater in small than in large worms, that it "decreases rapidly during the first stages of starvation and continues to decrease more slowly during several weeks, but that in advanced stages of starvation it increases" (40, p. 256). The advanced stages in which it is reported that the rate of oxidations increases are apparently later than the stages included in the experiments of the present writer, although it is hardly possible to compare the rates of starvation in two different species of Planaria under different conditions of observation. The data regarding differences in rate of oxidations in animals of different size and during the earliest stages of starvation are in agreement with those of the present paper.

These conclusions, it may be noted, are very different from either of the two former conclusions of Child which were based on *susceptibility* data. Child himself calls attention to these discrepancies and offers an explanation to preserve the theory that susceptibility is a measure of rate of oxidations. He recalls observations previously neglected, to the effect that during early starvation of Planaria, the susceptibility of the intestinal tract is different from that of the body wall, the former decreasing while the latter increases. The interpretation suggested is that

the ectoderm and body wall, which maintain their functional activity and subsist in part upon their own substance from the beginning, show a gradual increase in rate of oxidation during starvation, while the rate of oxidation in the alimentary tract undergoes rapid and marked decrease in the absence of food and probably still further decrease more slowly, as the reserves are used. Alimentary tract oxidation in well-fed animals is so large a part of the total oxidation that the decrease in activity of the alimentary tract in the absence of food brings about a decrease in total CO_2 production, in spite of the probable increase in CO_2 production in ectoderm and body well (40, p. 254).

In the absence of any experimental evidence that the oxidations in the body wall change with starvation and feeding, as Child suggests, rather than in accordance with the oxidations of the animal as a whole, the observed facts must be considered damaging to the theory that susceptibility is a measure of rate of oxidations. If the possibility that the oxidations in the body wall may change in a direction exactly opposite to the rate of oxidations of the whole animal is pressed, it must be borne in mind that the evidence which is cited in support of the theory, also, consists of measurements of the respiratory exchange of *whole animals*. Estimations of the total carbon dioxide production by Planaria in the Tashiro "biometer" have served as proof of the value of the susceptibility method. They were cited for this purpose in the last paper by Child (42, p. 381) although in the preceding paper it was reported that the biometer gave different results (as regards different conditions of nutrition) than the indicator method, so that "work with the biometer was discontinued because I came to believe that the method was not suitable for the material" (40, p. 250). However, whether reliance is placed upon the "biometer," the indicator method or the writer's Winkler method, the data obtained by these methods have given only the rate of respiratory metabolism of *whole animals*.

The distinction between the rate of oxidations in the animal as a whole and that in particular organs carries the question ultimately to the individual cells. Here we have the information that starving Paramecia are more susceptible to cyanide but have a lower rate of oxidations than fed Paramecia. The explanation suggested by Child is that during starvation in Paramecium, the ectoplasmic oxidations increase while the endoplasmic oxidations, which make up the major part of the whole, decrease. Is susceptibility in other animals such as Planaria to be attributed finally to conditions in the surface of the cell rather than to the oxidations of the cell as a whole? What does survival time tell us *with certainty* about oxidations?

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EXPERIMENTAL STUDIES OF THE URETER

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A large number of papers have been published dealing with the physiology of the ureter; the main conclusions may be summarized briefly.

Engelmann (1) making use of simple inspection described the peristalsis and anti-peristalsis proceeding with a certain velocity. On microscopic examination he found no ganglionic structures except in the lower part. He believed, therefore, that the contraction originates and travels in the muscle tissue itself without any relation to extrinsic or intrinsic nerves. Protopopow (2) also making use of ocular observation investigated the movements of the ureter in asphyxia and in different conditions of the circulation of the kidney and ureter. His experiments showed that an acceleration of the movements is caused by the distention of the ureter lumen, while a depression results from the ligation of the upper end. Concerning the innervation of the ureter he obtained a motor effect from the splanchnic nerve and a slight inhibitory result by stimulating the anastomosis between the inferior mesenteric ganglion and the plexus hypogastricus. In his work he tested the influence of a number of drugs upon the ureteral movements in situ. Atropin caused first an acceleration, later a depression; diuretin had no effect and caffeine stimulated the contractions in small doses, but depressed them in large doses.

Besides simple inspection by means of which Engelmann and Protopopow carried out their experiments, more recent investigators have studied the movements of the ureter either by recording intra-ureteral pressures or by using excised ureter strips.

Papers dealing with the pressure changes in the ureter lumen, by which its movements were studied, have been published by Fagge (3), Henderson (4), Berensnegowsky (5), Lucas (6), Sokoloff and Luchsinger (7).

Fagge found a motor effect of the hypogastric nerve upon the ureter;

Henderson and Berensnegowski separately concluded that a ureteral pressure does not depend entirely upon the contraction of the ureter. Lucas mentioned that the contractions are different in size and in number in various parts; the middle part showing relatively large and few contractions, while the upper part gives small and frequent waves of contractions. He also observed a complete prevention of the reflux of the bladder content into the ureter and described an action of the ureter for preventing by its peristalsis the accumulation of urine in the renal pelvis. Sokoloff and Luchsinger obtained contractions by the distention of the ureter lumen.

On the other hand some authors as Stern, Lucas (6), Roth (8) and Macht (9) made their experiments with ureter strips removed from the body.

Stern records some facts concerning the temperature relations and the proper nutrient solutions for the contractions of the excised ureter. Lucas tested the effects of various drugs; adrenalin, diuretin and barium chloride increased the tone and contraction, while chloroform, ether, chloral and magnesium sulfate exerted a final depressing effect preceded by a slight irritation. Roth stated that oxygenated Locke's solution of a certain composition is an excellent medium for the development of the contraction of the excised ureter. He observed two varieties of contractions occurring in a longitudinal strip, namely, large and small contractions. According to his experiments the small contractions predominated in the kidney third, while in the lower part large contractions were most frequently observed. Adrenalin and barium chloride caused an immediate increase in the tone and in the rate of the contraction, while apocodein depressed the activity of the ureter. From the effect of these drugs he assumed the presence of sympathetic nerve endings throughout the ureter.

Macht recently published a series of communications under the title of *On the pharmacology of the ureter*. He used the ureter ring preparation to study the behavior of the circular muscle coat. After suspending his preparation in normal Locke's solution he introduced several drugs such as adrenalin, ergotoxin, physostigmin, atropin, nicotin, morphin and various derivatives of morphin. From these experiments he obtained pharmacological proof that two sets of nerve endings are found in the ureter wall; one belonging to the sympathetics and the other to the parasympathetics. He also demonstrated the presence of ganglia throughout the ureter from the effect of nicotin.

1. CONTRACTION OF THE EXCISED URETER STRIP

a. Single strips

A number of observations on the contractions of the excised ureter in nutrient solutions at certain temperatures and effects of certain drugs upon their contractions have been described by such authors as Stern, Lucas, Roth, Macht and others. I have repeated these experiments under the same or different conditions.

Method. In this study I have generally used the excised ureter of the pig, because as Macht has stated, the pig's ureter has the most regular contraction and a longer vitality in artificial nutrient solutions. The material was obtained from a slaughterhouse in the neighborhood of the school. Pigs were bled to death and immediately after killing the ureters on both sides, connected with the kidneys and the bladder, were removed and placed in Locke's solution at room temperature. For longer preservation of their vitality, they were kept in Locke's solution on ice. Under these conditions striking contractions may be observed two days or more after removal. A very short strip (2 or 3 mm.) was cut off from the ureter to use as a ring after the manner of Macht, and also a longer section (10 to 20 mm.) as a longitudinal segment (circular fibers were opened). The former preparation registers the contraction of the circular muscle fibers and the latter represents that of the longitudinal fibers.

For the nutrient solution, Locke's solution of the following composition was employed almost exclusively in this work, because Roth found it the most efficient for the development of the spontaneous rhythm and the preservation of the vitality of the excised ureter of the dog.

Sodium chloride	9.0 gm.
Calcium chloride	0.24 gm.
Potassium chloride	0.24 gm.
Sodium bicarbonate	0.3 gm.
Dextrose	1.0 gm.
Distilled water	1000.0 cc.

The solution was made fresh for every experiment, and I could remove the sugar from the solution with very little deleterious effect upon the action of the ureter. Thus a ureter ring (or longitudinal strip) was placed in a bath of 20 cc. of Locke's solution, which was kept at a constant temperature of 38°C. The solution was also oxygenated by continual bubbles of oxygen gas. The ureter ring was

suspended between two hooks, of which the one was fixed at the base of the bath and the other was connected with the arm of a writing lever.

Character of the contraction of the ureter To demonstrate the character of the ureteral movements, the ureter rings were employed chiefly because the circular layer is mainly concerned in causing the peristaltic contractions. Every part of the ureter is capable of spontaneous contraction when placed in oxygenated Locke's solution at a temperature of 38°C.

The beginning of spontaneous contractions of the ureter ring after being placed in Locke's solution varies in the first place with the part of the ureter from which the ring is taken, and in the second place it depends upon the length of time after its removal from the body. The initial contraction starts immediately in the fresh ureter, while the ureter kept in the refrigerator for twenty-four or more hours begins to contract thirty minutes or even one hour after being placed in the bath.

The curve of contraction exhibits a steep ascending part rising almost vertically on a slowly revolving drum. The descending limb falls less steeply so that the contraction curve approaches gradually to the base line. Each contraction is followed by a pause represented as a horizontal line for a certain length. Sometimes the contractions follow one another closely, separated by no pause. But as a rule they do not fuse together, the descending limb reaching the level of the base line during the pause. Occasionally the contractions followed so closely, that there was partial fusion into a compound contraction. Finally there was observed a different kind of contraction in which there was a "plateau" at the summit of the curve. Such a curve has the form of an inverted U.

In spite of the different types of the contraction curves described above, there is noticed a striking similarity in each tracing made with a single ureter ring, placed in normal Locke's solution. In other words each ureter ring has only one kind of contraction curve from beginning to end.

The height and width of the curves also are the same within certain limits of time except when the contractions become gradually weaker due to fatigue.

The rate of the contractions varies in the various segments. Generally they are infrequent at first and then show a gradual increase to a certain rate, which is maintained for many hours even though becoming weaker in amplitude.

The amplitude of the contractions of the several segments also varies. This variation seems to depend to a large extent upon the structure of the muscle layer of the different parts as described elsewhere.[1]

Effects of certain drugs. The above description applies to the contraction of the ureter ring under normal physiological conditions. An attempt was made to study the action of various drugs upon the contraction of the ureter rings.

The definite finding that nerve plexuses and ganglia exist throughout the ureter wall has been described in the anatomical part of my work. Previous investigators are not in accord as to the innervation of the ureter; some maintaining a supply only from the thoracico-lumbar autonomies, the so called "true sympathetic," and others finding two sets of nerve fibers, namely the sympathetic and the autonomic nerve supply of sacral root origin, the so-called "parasympathetic."

For the purpose of investigating these nerve supplies, adrenalin and ergotoxin on the one hand and physostigmin and atropin on the other hand were employed, following the general belief that the first two act upon the sympathetic nerve endings while the latter two are excellent agents for revealing the presence of the parasympathetic nerve endings.

Experiments with these drugs were carried out according to the following schema.

1. Adrenalin (in small to large doses).
2. Ergotoxin (in small to large doses).
3. Adrenalin (in large doses) and then ergotoxin (in large doses).
4. Ergotoxin (in large doses) and then adrenalin (in large doses).
5. Physostigmin (in small to large doses).
6. Atropin (in small to large doses).
7. Physostigmin (in large doses) and then atropin (in large doses).
8. Atropin (in large doses) and then physostigmin (in large doses).
9. Atropin (in large doses) and then adrenalin (in large doses).
10. Ergotoxin (in large doses) and then physostigmin (in large doses).
11. Nicotin (in small to large doses).

Adrenalin even in a small dose stimulates the contraction of the ureter.

When a minute quantity of adrenalin (1 drop of 1: 10,000 solution) was added to the bath consisting of 20 cc. Locke's solution and in which a ureteral ring preparation had been suspended, a marked effect was soon noticed. The spontaneous contractions of the ring showed

[1] To appear shortly in the Journal of Urology.

a striking increase in rate, although no change in tonus and amplitude was obtained with such a small amount of adrenalin. A ureter which had been kept on ice in Locke's solution for one day or more and had shown no contractions when suspended at room temperature, began to contract rhythmically.

The larger doses (3 to 8 drops of 1: 10,000 solution in 20 cc. Locke's) caused an immediate increase of the rate, together with a slight increase in the tone. The amplitude of the contractions showed also a very slight increase. Thus adrenalin exerts an influence on the ureter ring in rate, tonus and amplitude in proportion to the increase in concentration.

Ergotoxin in relatively small doses (0.5 to 1 mgm. in 20 cc. Locke's solution) caused an increase in the tone and in the rate of contraction.

FIG. 1. Portion of graphic record obtained in experiment of November 6, 1918, with a ureter ring made from the middle of the pig's ureter in 20 cc. Locke's solution. *1*, spontaneous contractions; *2*, the effect of addition of 1 drop of adrenalin (1: 10,000); *3*, the effect of addition of 2 drops of adrenalin (1: 10,000); *4*, the effect of addition of 3 drops of adrenalin (1: 10,000); *5*, return to Locke's solution.

This effect may be regarded as a primary stage of stimulation. With increasing doses the tonus increased correspondingly, but the rhythmic contractions disappeared after a certain period of time, so that the ureter ring passed into a condition of tonic contracture. I did not find how long and how far this contracture would go on, as I put the ring again in the normal Locke's solution before observing a relaxation. But there was no relaxation throughout a rather long interval of time while under the influence of ergotoxin.

These experiments with adrenalin and ergotoxin indicate the presence of sympathetic nerve endings in the ureter wall. The effects of these drugs are distinctly different in several parts of the ureter, as will be described later.

Physostigmin in small and large doses (1.0 to 15 mgm. in 20 cc. Locke's solution) and pilocarpin in small doses (30 mgm. in 75 cc.

Locke's) increased the contractions in rate and also in tonus. Atropin in small doses exhibited sometimes a primary stage of stimulation, so that the ureteral contractions increased in rate. But the amplitude of the contractions decreased gradually in every case, and after a longer or shorter interval movements ceased entirely. There was found, however, no sudden disappearance of the contractions of the ureter even after the introduction of a rather large amount of atropin (15 mgm. in 20 cc. Locke's).

Fig. 2. Experiment of November 6, 1918, on a ureteral ring from the middle third of a pig's ureter. *1*, the effect of addition of 0.5 mgm. ergotoxin; *2*, the effect of addition of 1.0 mgm. ergotoxin; *3*, the effect of addition of 5.0 mgm. ergotoxin; *4*, return to Locke's solution.

Fig. 3. Portion of graphic record obtained in experiment of November 8, 1918, with a ring from the lower part of the middle third of the pig's ureter in 20 cc. Locke's solution. *1*, the effect of addition of 1.0 mgm. physostigmin; *2*, the effect of addition of 3.0 mgm. physostigmin.

Inasmuch as physostigmin and atropin are regarded as exercising a selective action on the parasympathetic nerve endings, these latter results seem to furnish pharmacological evidence for the presence of sacral autonomic fibers. This conclusion is corroborated by the results obtained from direct stimulation of the nerves, as will be described below.

The effect of combinations of these drugs upon the excised ureter is of interest as throwing more light on the question of its innervation.

As I have shown above, ergotoxin antagonized the action of adrenalin, but had no effect on the stimulating effect of physostigmin. In other words the pressor action of physostigmin was not affected by previous administration of ergotoxin, while adrenalin after ergotoxin was not able to cause contractions of the ureter ring. Moreover in experiments in which atropin and adrenalin were introduced successively after a certain interval of time, it was found that atropin did not interfere with the action of adrenalin although it inhibited entirely the effects of physostigmin.

Fig. 4. Portion of graphic representation traced by a ring from the middle of the pig's ureter in experiment of November 7, 1918. Preparation was placed in 20 cc. Locke's solution, and shows the final inhibitory action of atropin. *1*, the effect of addition of 1.0 mgm. atropin; *2*, the effect of addition of 5.0 mgm. atropin; *3*, the effect of addition of 7.0 mgm. atropin; *4*, the effect of three drops of adrenalin (1: 10,000) after atropin.

Fig. 5. Portion of graphic record obtained in experiment of November 6, 1918, with a ring preparation from the middle of the pig's ureter in 20 cc. Locke's solution. *1*, no effect of addition of 3 drops of adrenalin (1: 10,000) after ergotoxin; *2*, the effect of addition of 5.0 mgm. physostigmin after ergotoxin.

These facts may be accepted as showing that these drugs exert their action on different structures in the ureter wall, in accordance with the view outlined above.

In addition it was thought desirable to examine the action of nicotin upon the ureter ring, since it is one of the accepted agents used to paralyze peripheral ganglion cells. Its action is to stimulate first and then paralyze. Small doses of nicotin (3 drops of a 1: 100 solution in 20 cc. Locke's) caused an increase in the contractions, especially in

amplitude, and medium doses (8 drops of a 1: 100 solution in 20 cc. Locke's) exerted the same effect in a stronger measure, but caused a little decrease in the rate of contractions.

Large doses (1 drop of the undiluted solution in 20 cc. Locke's) paralyzed the contractions immediately.

Thus the effect of nicotin upon the excised ureter indicates the existence of ganglion cells in the ureter itself. It also points to the presence of ganglionic structure throughout this organ, since experiments with all parts of the ureter gave positive results of the kind described.

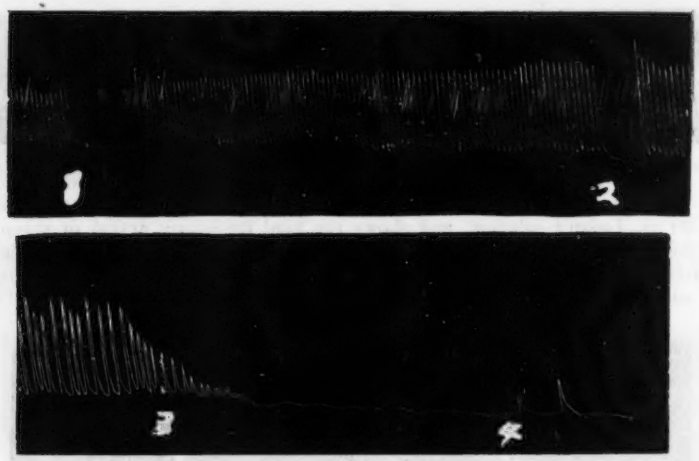

Fig. 6. Portion of graphic record obtained in experiment of November 7, 1918, with a ring from the middle part of the pig's ureter in 20 cc. Locke's solution, showing the action of nicotin in increasing doses. *1*, three drops of nicotin (1: 100); *2*, eight drops of nicotin (1: 100); *3*, one drop of concentrated nicotin; *4*, return to Locke's solution.

b. The use of three strips to compare the contractions of three different parts

As pointed out above, the contraction of the ureter ring is markedly different in the several parts of the organ. These differences were observed not only under normal physiological condition, but also after the introduction of drugs in the bathing solution. In the present experiments three ureter rings made from the upper, the lower and the

middle portions were selected for the purpose of comparing their contractions, since in these regions the greatest difference in the structure of the muscle coat is found. They were placed in a glass beaker containing 75 cc. oxygenated Locke's solution. Three straw levers, which were of the same length and of the same weight, were connected with the rings. Tracings of these levers were obtained on a revolving drum. In this way curves of the contractions of the three parts under similar conditions can be obtained simultaneously. It should be stated that the ureters used in these experiments were not perfectly fresh but had been kept for some hours (3 to 24) in cold Locke's solution.

The three rings did not begin to contract at the same time. The upper ring (the ring made from the upper part of the ureter) began to contract usually immediately after placing it in the bath, and the middle ring followed after a longer or shorter period of time, while the lower ring was quiescent in most of the experiments in the normal Locke's solution for a number of hours.

This finding indicates that the irritability of the ureter varies in different parts; the upper end being the most susceptible to stimulation, and the lower parts showing a gradual decrease in irritability.

Further there were noticed marked differences in the amplitude of the contractions. The upper ring exhibited small rhythmic contractions, while the middle ring gave much larger curves, so that the height of the contractions was many times greater than that of the upper ring. The spontaneous contractions of the lower ring, on the contrary, were very small, much smaller than those of the upper ring. This difference in amplitude of the contractions depends upon the amount and the course of the muscle fibers. In other words, the amplitude of the contraction is proportional to the quantity of the circular muscle fibers.

The rate of the contractions also varied definitely in different parts of the ureter. The highest rate was found in the upper ring, while in the middle and the lower ring the rate was slower to about the same degree.

The difference in the effects of certain drugs on these three rings is most interesting. Adrenalin caused an increase in the contractions of the upper and middle rings in both rate and amplitude; its effect being stronger on the upper ring. On the lower ring, on the contrary, adrenalin had only a very small effect, causing a minute increase in the rate or sometimes no reaction at all, even in large doses. The re-

Fig. 7. Graphic record obtained in experiment of November 22, 1918. Spontaneous contractions of three rings of a pig's ureter, which had been kept for hours in a cold Locke's solution. Upper record, contractions of a ring from the upper part; second record, those of a middle ring; third record, movements of a lower ring; fourth record, base line.

sponse of the middle and lower rings to adrenalin started immediately after introducing the drug, while the upper ring began to react after a certain period of time. I cannot find a probable explanation for this result.

Physostigmin exerted its effect on the middle and lower ring, producing an increase in the rate and amplitude. Vigorous contractions were evoked in a quiescent lower ring, which had remained motionless for many hours after being placed in Locke's solution. In the upper ring, however, physostigmin had either a slight effect or no pressor action at all. In regard to the beginning of the contraction there was found a

Fig. 8. Portion of graphic tracing obtained in experiment of November 21, 1918. Three rings were suspended in 75 cc. Locke's solution, to which adrenalin was added (8 drops of 1: 10,000 solution). Upper record, the contractions of the upper ring; second record those of a ring from the middle part; third record, movements of the lower ring; forth record, base line. *1*, addition of adrenalin.

reversed order compared with that of adrenalin. Contraction developed immediately, if at all, in the upper ring while in the middle and lower rings contractions appeared only after a certain interval.

Comparing the findings of the experiments carried out with three rings, it is seen that adrenalin has a stronger influence at the upper part of the ureter, while physostigmin exerts a more marked effect on the lower part.

The middle part reacts to these two drugs almost in the same degree.

These facts indicate that the upper part has a nerve supply from the sympathetics chiefly, while the larger part of the nervous element in

the lower part belongs to the parasympathetic system. In the middle part there exist both sets of nerve fibers, seemingly in the same quantity.

Comparison of the effects of adrenalin and physostigmin on the tonus of the three rings develops an interesting difference. Adrenalin caused an increase of the tonus in the upper and middle rings only to a slight degree. On the contrary, physostigmin produced tonic contractions in the middle and lower rings much more striking than the tonus of the upper and middle rings produced by adrenalin. This and other facts seem to indicate a difference between these two sets of nerves in the ureter in respect to their physiological functions; the action of the

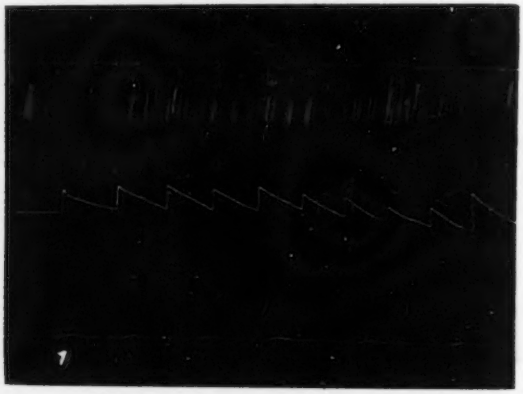

Fig. 9. Portion of graphic tracing obtained in experiment of November 21, 1918. Three rings of a pig's ureter were placed in 75 cc. Locke's solution, to which 15 mgm. physostigmin was introduced, *1*. Upper record, the contractions of the upper ring; middle record, those of the middle ring; lower record, movement of the lower ring.

sympathetics has an evident relation to the rhythmic contractions of the ureter, while the parasympathetics may act rather as a pressor nerve for heightening the tonus.

2. EXPERIMENTS UPON STIMULATION OF THE EXTRINSIC NERVES

After administration of 1 grain of morphin a dog of medium size was fixed on the holder. The animal having been anesthetized by ether and tracheotomized, the abdominal cavity was opened along the linea alba from the xyphoid process to the symphysis pubis. For the pur-

pose of making a sufficient space to find the left splanchnic nerve in the region above the suprarenal gland, another incision was made on the left abdominal wall along the lower border of the last rib. The intestines were drawn to the right side and the viscera, as well as the rest of the abdominal wall, were covered with warm towels. In this way the left ureter could be seen completely from the kidney to the bladder. For a little while after opening the abdominal cavity the regular peristaltic movements were noticed at the rate of 3 to 5 per minute. They disappeared, as a rule, in a few minutes but in some cases might continue for hours. In some dogs the ureters remained motionless even immediately after opening the abdomen.

The method employed in obtaining graphic records of the ureteral movements consisted in connecting the lumen of the ureter with a water manometer, the undulations of the column of water being then transmitted by means of a piston recorder to a revolving drum.

The connection with the ureter was made by the following method. After opening the bladder cavity by a long sagittal incision, a small cannula with a thin tip was introduced into the orifice of the left ureter. To avoid losing the cannula from the orifice and to keep the connection water-tight, four weak rubber bands were used, one end being hooked to the mucosa of the bladder in the neighborhood of the orifice and the other end being tied to the glass cannula, some distance from the orifice.

By this method the ureter was retained in its normal connection with the bladder, and no injury was inflicted upon the nerve and blood supplies which ascend up the ureter from the bladder. To prevent an increase of the intra-ureteral pressure due to the urine secretion of the kidney, the upper end of the ureter was squeezed with a weak artery-clamp. When all preliminary arrangements had been made the intra-ureteral pressure was raised by injecting Locke's solution through the cannula into the ureter lumen, which was thereby distended to a certain degree.

PROTOCOLS OF TYPICAL EXPERIMENTS

All of the experiments of the present series were made on dogs. The results of three typical experiments are reported.

Experiment 3. December 11, 1918. The preliminary operation described above was made. After tying the trunk of the left splanchnic nerve in the region above the suprarenal gland, the nerve was cut and the distal stump of the nerve was stimulated with induction currents for half a minute. As a result

of this stimulation there occurred after a quarter of a minute three contractions in the previous quiescent ureter. The stimulation was repeated with the result of one contraction for each stimulation. But the interval between the stimulation and the appearance of the contraction became gradually longer, as the stimulations were repeated. After the seventh stimulation there was no response owing probably to a fatigue of the nervous mechanism. By pulling the splanchnic nerve also there occurred a series of contractions (mechanical stimulation).

Fig. 10. Portion of graphic record obtained in experiment of December 11, 1918. Upper record, contractions of the ureter from stimulating the splanchnic nerve; lower record, time record in minutes. *1, 2, 3, 4, 5, 6, 7*, stimulations of the nerve.

The left pelvic nerve was dissected out in the pelvic cavity and was then ligated and cut. On the distal side of the nerve many branches making up a plexus spread in the form of a fan over the side of the bladder. The uppermost one of these branches, which seemed to be larger than the other fibers, was stimulated electrically. There was observed, however, no contraction of the ureter. But when the electrodes were brought under the nerve plexus and touched a certain branch, there appeared suddenly contractions, which continued rhythmically for a short time, giving a series of contraction curves.

An interesting event, which indicated clearly that mechanical stimulation is able to cause contractions of the ureter, happened during this experiment.

Fig. 11. Portion of graphic record obtained in experiment of December 11, 1918. Upper tracing, contraction of the ureter from mechanical stimulation (squeezing) of the pelvic nerve, *1*. Lower record, time record.

I lost the distal stump of the pelvic nerve, as its end escaped from the ligature. When I found it after a little while and squeezed it with an artery forceps, there occurred a series of contractions. The contractions followed so closely, especially in the middle section of the series, that they fused into an incomplete compound contraction, showing an increase of the tonus of the ureter.

Experiment 6. December 18, 1918. The operation and the arrangement of the apparatus were the same as in the preceding experiment. At first the ureter remained quiescent. But electrical and mechanical stimulation of the splanchnic nerve caused a contraction or a series of contractions immediately.

After a few stimulations of the splanchnic nerve the ureter showed somewhat irregular spontaneous contractions, which continued for hours. Stimulation of the splanchnic nerve had an effect of making the rhythm more regular.

Stimulation of the pelvic nerve gave also contractions of the ureter. The curves of contraction, however, were lower and wider than in the case of the contractions due to stimulation of the splanchnics.

Fig. 12. Portion of graphic record obtained in experiment of December 18, 1918. Upper tracing, contractions of the ureter from stimulating the splanchnic nerve mechanically, *1*, and by induction current, *2*. Lower record, time record.

In regard to the number of contractions following a single stimulation of either the splanchnic or pelvic nerves the result varied within wide limits, sometimes only a single contraction appearing either immediately or after a short time, or sometimes a number of contractions succeeding each other.

Comparing the contractions caused by stimulating the splanchnic and pelvic nerves, a difference was observed. The contractions caused by the stimulation of the splanchnic nerve were quicker and stronger, while the contractions caused by stimulating the pelvic nerve were slower and weaker. This difference seems to indicate that the two sets of nerves may have different actions upon the ureteral movements.

Further there was noticed a number of vigorous contractions by stimulating a nerve trunk, which was found under the left kidney. It was situated 2 or 3 cm. below the origin of the renal artery, over the ventral side of the aorta.

The contractions continued for several minutes at the rate of 4 to 8 per minute. But the nerve was not dissected out to find its origin.

Experiment 11. January 8, 1919. The ureteral movements caused by stimulating the splanchnic and pelvic nerves were just the same as in the previous experiments. In this experiment a very delicate branch from the inferior mes-

enteric nerve was dissected out. It had its origin from a point of the inferior mesenteric nerve in the neighborhood of the inferior mesenteric ganglion, and divided soon after leaving the inferior mesenteric nerve into two or more branches, the upper one ascending somewhat and the lower branch taking a more descending direction. These final branches evidently had their terminations in the ureter wall. Stimulation of this branch of the inferior mesenteric nerve with induction currents caused an immediate and marked diminution in the rate of a series of spontaneous contractions which the ureter had been giving.

After cessation of the stimulus the rate of contractions increased markedly, so that it became one and a half times more than that during the spontaneous contractions.

In regard to the amplitude of the contractions, however, there was observed almost no change during and after stimulation of this nerve branch. From this finding the small branch from the inferior mesenteric nerve may be regarded as an inhibitory nerve to the movements of the ureter. Protopopow states

Fig. 13. Portion of graphic tracing obtained in experiment of January 8, 1919. Upper record, inhibition of the contractions of the ureter from stimulating the ureteral branches of the inferior mesenteric ganglion. *1*, point of beginning of stimulation; *2*, end of stimulation. Lower record, time record in minutes.

that stimulation of the anastomosis between the inferior mesenteric ganglion and the hypogastric plexus caused usually no acceleration, but sometimes rather a slowing of the ureteral contractions. When the hypogastric nerve was stimulated electrically, there was a very weak inhibitory result.

This nerve was cut at the middle and the distal stump was stimulated, causing no change in the movements of the ureter. On the other hand, stimulation of the proximal stump caused clearly an inhibitory effect of the ureteral movements though in a slight degree. These facts accord with Protopopow's findings and seem to indicate that the electrical stimulation reached the inferior mesenteric ganglion through the hypogastric nerve and presumably as an axon reflex influenced the ureteral contractions through the nerve branches passing from the inferior mesenteric nerve to the ureter. Sometimes there occurred evidence of tonic contractions after stimulation of the branch was stopped.

Painting and intra-ureteral injection of adrenalin, atropin and other drugs caused their respective effects in the same manner on the ureter in situ as in ring preparations, but to a slighter degree.

As a result of these three typical experiments and others which gave similar results light is thrown upon the important question of the innervation of the ureter. The motor effect of stimulation of the splanchnic nerve indicates evidently that there are nerve endings of the thoracico-lumbar (sympathetic) outflow in the ureter. As this fact has been stated by many different observers we may assume that it can be accepted without question. In almost all of my experiments (nos. 3, 4, 6, 7, 8, 9, 10, 11, 12, 14, 15) this result was obtained.

In regard to the innervation of the ureter by the pelvic nerve, in other words the presence of parasympathetic nerve endings, there has been some difference of opinion. In most of the present experiments (nos. 3, 4, 6, 8, 9, 11, 12, 14) definite evidence was obtained of the existence of the parasympathetic motor neurones, which come up to the ureter through the connector nerve fibers of the sacral outflow, i.e., the pelvic nerve. Some objections may be made against the experiments on the ground that the electrodes must be brought so near to the ureter that the currents might escape into the ureter through the tissues which accompany the fibers of the pelvic nerve.

But the fact that mechanical stimulation such as squeezing the nerve, as in experiment 3, also caused a series of vigorous contractions, can be regarded as indisputable evidence that the pelvic nerve carries motor fibers to the ureter. This result accords also with the results which were obtained pharmacologically with the ring preparations of the excised ureter, as described above.

The method of experimentation described in this section did not throw any light upon the relative distribution of the sympathetic and parasympathetic fibers. The conclusion in regard to this point rests upon the pharmacological results already described.

DISCUSSION

On the peristalsis of the ureter. All smooth muscle tissues possess the power to contract, but they need to be in close continuity with nerve elements for the development of the contraction. The ureter has a well developed muscle coat, and within the ureter wall there is found a complete nervous system composed of a network of fibers with many ganglion cells as shown by many authors and confirmed in my study of the histology of this organ. According to my finding there are two nerve plexuses situated in the outer fibrous coat and submucosa immediately embracing the muscle layer over its outer

and inner sides. From the close situation of these nerve plexuses to the muscle layer we may infer that the contractions of the ureter are due to this intrinsic nervous system. That the ureter is capable of contracting spontaneously without the action of extrinsic nerves, is demonstrated by the spontaneous contractions of excised pieces when kept in Locke's solution at a temperature of 38°C.

In regard to the direction of the contraction wave of the ureter, which begins at the upper end and proceeds toward the bladder in the form of a peristalsis, the author finds an immediate explanation in the experiment in which three ring preparations made from different parts of the ureter were observed to contract after different periods of time; the upper end being most responsive to a stimulus and the responsiveness decreasing gradually toward the lower end. In the normal condition in situ the contraction wave develops at the upper end on account of the greater irritability at that end, favored perhaps also by the mechanical pressure of the secretion.

On the innervation of the ureter. The innervation of the ureter has been investigated by many authors, whose statements are quite divergent. Langley and Anderson (10) dissected out three ureteral branches from the hypogastric nerve in the cat. Protopopow suggested that the splanchnic nerve augmented the peristalsis of the ureter in the dog. Fagge and Stern observed an accelerating effect of the hypogastric nerve. Elliot (11) also described a slight movement of the ureter by stimulating the hypogastric nerve.

Gaskell (12) in his monograph on *The involuntary nervous system* mentions that the segmental ducts, namely the uterus, the ureter and the vas deferens, possess both motor and inhibitory fibers, which arise from nerve cells belonging to the thoracico-lumbar outflow, but adds that there is no evidence that these structures are connected in the slightest degree with the pelvic nerve. He states also that the nerve cells with which the sympathetic nerve fibers are connected are placed in the ureter itself. Gaskell's argument for the innervation of the ureter by the sympathetics only rests largely upon embryological considerations.

In my experiments I found pharmacologically, on the one hand, the presence of both the sympathetic and parasympathetic nerve endings. On the other hand, I confirmed by stimulation the innervation of this organ by both the splanchnic and the pelvic nerves. There is, therefore, no doubt of the existence of sympathetic and parasympathetic nerve endings in the ureter wall, connected with the preganglionic fibers of

the thoracico-lumbar and sacral outflow (i.e., the splanchnic and pelvic nerves) respectively. In two cases small nerve filaments were followed anatomically from the pelvic nerve to the ureter.

Delicate filaments given off from the inferior mesenteric nerve and going to the ureter were found on stimulation to exert an inhibitory effect.

On the prevention of the reflux of the bladder content into the ureter lumen. It is an important problem as to whether or not the bladder content can flow back into the ureter under normal conditions. As a matter of fact various clinicians describe an ascending nephritis, which follows infection of the bladder. If such is the case there is some doubt as to whether the deleterious condition in the bladder may have an effect on the kidney by direct transmission through the ureter, or in some indirect way.

Some urologists as Petit, Hallé, Sappy, Zuckerkandle and Tuchman, and the physiologist, Sigmund Mayer, have stated that the normal closure of the ureter is competent to prevent a reflux. Harrison, from his clinical observation, believed that regurgitation can only be brought about by very gradual means, not by a sudden flow into the ureter. Guyon and Albarran obtained the same result as Harrison in their experimental work. On the other hand, most of the experiments of Lewin and Goldschmidt (13) have indicated the possibility of a reflux even in normal conditions, but Lucas claims that the normal ureterovesicular valves can wholly prevent the reflux of the urine.

As a result of my experiments I find that normally there is no reflux into the ureter, although from my anatomical findings and those of many authors there is found no special structure of the nature of a sphincter at the end of the ureter.

In some of my experiments on dogs and pigs the bladders were removed with a small adjacent part of the ureters and urethras. The bladder was gradually distended by salt solution to the greatest degree, but there was no drop of leakage from the ureter stump; and the bladder thus distended could be kept for days. This fact indicates that the bladder content may not flow back into the ureter lumen even with fullest distention of the bladder. After destroying all muscles of the bladder wall, which cover the intraparietal part of the ureter, a slow leakage was produced through the ureter. The prevention of a reflux is referable to a physical mechanism to a large extent. The distended bladder muscles over the ureter press the ureter together closely and moreover the slit-like opening of the ureter tends to

close the opening so that even after destruction of the bladder musculature the leakage back into the ureter is very slow.

In addition to these physical mechanisms the prevention of a reflux is probably aided by a more distinctly physiological activity. A layer of longitudinal muscle is found on the outer side of its intraparietal part, while the Waldeyer's ureter sheath covers the medial side of the small section just above the intraparietal part. The contraction of the former tends to close the orifice, and the contraction of the latter causes the lip-shaped upper rim to protrude. These mechanisms thus assist in preventing the reflux of urine into the ureter.

CONCLUSIONS

1. The excised ureter gives spontaneous contractions in oxygenated Locke's solution. The contractions vary in rate and character with different individuals.

2. The amplitude and rate of the spontaneous contractions vary also in different parts of the ureter. The contractions of the middle part are large and slow, those of the kidney end are lower and faster, while at the bladder end the contractions are very small in size and slow in rate. These differences so far as size is concerned are correlated with the different thickness of the circular muscular coat which has its largest development in the middle section.

3. Adrenalin and physostigmin stimulate the movements of the ureter.

Adrenalin affects the upper portion of the ureter more strongly while physostigmin gives a more distinct reaction on the lower portion.

4. Ergotoxin in large doses antagonizes the action of adrenalin but has no influence upon the action of physostigmin. With atropin in large doses the opposite reaction holds; it antagonizes the action of physostigmin but not of adrenalin. In accordance with the usual interpretation, these reactions indicate the presence in the ureter of sympathetic and parasympathetic fibers.

5. Nicotin in large doses paralyzes the movements of all parts of the ureter, thus corroborating the histological finding of ganglion cells along the whole length of the ureter.

6. The irritability of the ureter as displayed by the rhythmic contractions of isolated rings in Locke's solution is greatest at the upper end and diminishes gradually toward the bladder.

7. The action of adrenalin and ergotoxin is most marked at the upper end of the ureter, while the characteristic effects of physostigmin

and atropin are displayed especially by rings taken from the lower end. The middle section reacts somewhat equally to the two sets of drugs. It is inferred from these facts that the upper end is innervated chiefly by sympathetic fibers, and the lower end by parasympathetic fibers, while in the middle there is an approximately equal distribution of the two sets of fibers.

8. Stimulation of the peripheral end of the splanchnic nerve causes the development of contractions in a quiescent ureter. Stimulation of the peripheral stump of the pelvic nerve also causes the development of contractions in the ureter.

9. The ureteral muscles receive inhibitory fibers by way of the communicating branch to the inferior mesenteric ganglion. The action of these fibers is to slow the rhythm of the ureteral contractions.

10. The prevention of a reflux of the bladder content into the ureter is complete under normal conditions. The closure is effected mainly by a physical mechanism, but is assisted by the physiological activity of the musculature at the intraparietal portion of the ureter.

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